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THE AMERICAN REVIEW OF TUBERCULOSIS

JOURNAL OF
THE NATIONAL TUBERCULOSIS ASSOCIATION

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VOLUME XIII
JANUARY - JUNE, 1926

10635

PUBLISHED MONTHLY
AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
BY THE NATIONAL TUBERCULOSIS ASSOCIATION

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TUBERCULOSIS OF THE PLEURA

An Anatomical Study[1,2]

WILLIAM SNOW MILLER

The lung is enveloped by a serous membrane, the *pleura pulmonalis*, which is continuous with the *pleura costalis* lining the thoracic cavity. The structure of the pleura varies in different animals. In the human lung it consists of four layers. Named from without in they are the mesothelial, the submesothelial, the elastic (sometimes termed the *elastica externa*) and the areolar layer. There is an inner layer of elastic fibres (sometimes called the *elastic interna*), but these fibres belong more properly to the alveoli which are situated directly beneath the pleura, than to the pleura itself (fig. 1).

The mesothelium consists of a layer of thin, flattened cells which have an oval nucleus situated near the centre of each cell. Openings which have been termed *stigmata* and *stomata*, depending on their size and position, have been described as being present in this layer; by means of these openings the pleural cavity is said to be in direct communication with the pulmonary lymphatics. That these openings (they have also been described in the mesentery and omentum) are *artifacts* and not natural, preformed openings, has been demonstrated by the work of Miller, Meyer, von Ebner and Walters.

The submesothelial layer has an average thickness of 8.5μ, and is composed of collagenous fibres, a few fine elastic fibres, which are derived from the underlying elastic layer, and a delicate network of reticulum, which is most pronounced just beneath the mesothelium.

The elastic layer averages 5.625μ in thickness, and is composed almost exclusively of elastic fibres which form an interlacing network. Bundles of collagenous fibres pass through the openings in this network from the areolar layer to the submesothelial layer. There is also a small amount of reticulum present. The fibres of this layer send off delicate

[1] From the Anatomical Laboratory of the University of Wisconsin. Aided by a grant from the National Tuberculosis Association.

[2] Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

branches into the submesothelial layer, and anastomosing branches into the areolar layer.

The areolar layer is the one in which the blood vessels, lymph vessels and nerves are situated. It has an average thickness of 60μ, and is made up of coarse bundles of collagenous fibres, scattered elastic fibres which anastomose on the one hand with the fibres in the elastic layer and, on the other hand, with the elastic fibres in the walls of the underlying alveoli, and of a network of reticulum which is especially well developed about the blood and lymph vessels.

In the human lung prolongations of the areolar layer of the pleura extend into the lung and form the septa which mark out the secondary lobules. This characteristic does not belong exclusively to the human lung; it is found in the lung of the ox, pig, sheep and many other animals; on the other hand septa are not found in the lung of the dog, cat, rabbit or rat.

The presence or absence of secondary lobules, with their accompanying septa, determines the source of the blood supply of the pleura. In those animals in which septa are present the bronchial artery passes along the septum and is distributed to the pleura and to the septum, while in those animals (so far as my studies have gone) in which there are no septa the pulmonary artery supplies the pleura. In the lungs of those animals which possess septa the pleura is of considerable thickness, while in those in which septa are absent the pleura is often of exquisite thinness; as, for example, in the rabbit. As septa are present in the human lung it must, therefore, be classed as a lung with a thick pleura.

Anatomically, tubercles may be situated in the submesothelial layer or in the areolar layer. At first they exist as discrete, microscopic tubercles; but as they progress in development the entire pleura and the underlying lung substance may become involved, or growth may take place in the opposite direction and the pleural cavity may be invaded. In some instances there is a generalized tuberculosis of the pleura without the formation of discrete tubercles. The lung substance underlying the pleura may be tuberculous without primarily including the pleura; but with the extension of the tuberculous process the pleura may become involved.

Although the main facts of the following case, and the accompanying illustrations have been previously published, I repeat them because

they furnish the most typical example of a tubercle developing in the submesothelial layer that I have found. I extract the following from the protocol of the necropsy.

Negro. Male. Thirty years old. Ill nine months. Extensive tuberculosis with cavities in the upper lobe of the left lung. The right lung and the lower lobe of the left lung contained fine miliary tubercles. In the upper right lobe the tubercles were more confluent than elsewhere in the lungs. There was a slight fibrinous pleurisy. Beneath some of the flakes of fibrin there were tubercles which appeared smaller than elsewhere.

Situated within the mesothelial layer of the pleura there is a single microscopic tubercle containing two giant cells (fig. 2). This has given rise to a planoconvex disk, 0.15 mm. in diameter, which is bounded externally by the mesothelium and internally by the elastic layer of the pleura. The remaining layers of the pleura and the underlying pulmonary substance are not involved in the tuberculous process. The outline of the tubercle is more clearly defined in figure 3 in which the reticulum has been stained. The heavily stained fibres which are situated beneath the tubercle are bundles of collagenous fibres which belong to the areolar layer of the pleura. In figure 4 the elastic fibres have been stained, and it clearly shows that the tubercle is limited to the submesothelial layer. Distended blood and lymph vessels can be distinguished in the areolar layer of the pleura and in the pulmonary substance underlying the pleura.

Although the protocol of the necropsy which accompanied this specimen did not specifically state that there was a pleuritic effusion, the presence of flakes of fibrin scattered over the surface of the lung warrant the supposition that it was present. Since the submesothelial layer contains neither blood vessels nor lymph vessels, there seems to be no doubt that this tubercle had its origin from bacilli which found their way into the pleural cavity from some other source and had reëntered the pleura from the pleuritic effusion. The points of special interest in these sections are (1) the absence of any exudate over the tubercle, although flakes of fibrin were elsewhere present; (2) the tubercle has not extended beyond the submesothelial layer; and (3) the absence of any lesion in the underlying pulmonary substance.

That the elastic layer of the pleura may be of assistance in marking out the extent of lesions within the pleura, was pointed out by W. C. White in 1901, but before I had knowledge of his publication I had called attention to this characteristic in the AMERICAN REVIEW OF TUBERCULOSIS, vol. vii, p. 153.

For a typical case in which tubercles have developed in the areolar layer of the pleura, I am indebted to Dr. Kennon Dunham. (For an X-ray chest plate before death, and an X-ray lung plate after death, see case 1168 described by Dunham and Skavlem in the AMERICAN REVIEW OF TUBERCULOSIS, vol. v, p. 304.) The sections are from the area marked *4*, in their figure 12, and are taken from a deeper portion of the area shown in their figure 13.

In figure 5, which is a transection through its greatest diameter, a microscopic tubercle can be seen situated beneath the endothelium lining a lymph vessel, and projecting into the lumen of the vessel. Within this tubercle there are two giant cells; the larger cell is cut in such a plane that numerous nuclei appear in its section, but, owing to the oblique position of the smaller cell, only a few nuclei can be seen. The outer surface of the pleura is covered with a fibrinous exudate which is beginning to undergo organization. On the inner (pulmonary) side of the lymph vessel, there is a dilated branch of a pulmonary vein. Deeper in the section, and situated just beneath the pleura, there is a group of miliary tubercles, and still deeper there is an area of tuberculous bronchopneumonia that has undergone caseation in its centre.

The tubercle shown in figure 6 is from the same series of sections as figure 5, but it is situated some distance to one side, and below, the tubercle there shown. It has attained a greater development than the one in figure 5 and, like the latter, is situated in the wall of a lymph vessel. The blood vessels in the immediate neighborhood of this tubercle are not as distended as in figure 5, and the pulmonary structures lying immediately under the pleura are not involved in a tuberculous process. This may account for the blood vessels being more normal in size. In both figures 5 and 6 the areolar layer is increased in thickness, and in figure 6 it is oedematous.

The following abstract is taken from the history which accompanied the specimen from which figure 7 was obtained:

> Male. Negro. Age 30 years. Clinical history extends over five months. Cough with expectoration of purulent matter. Progressive loss of weight and night-sweats. Sputum positive. X-ray shows the heart and mediastinal contents displaced to the left. Both the upper and lower left lobes show patchy mottling of evenly distributed densities. No protocol of the necropsy accompanied the specimen.

Sections of the left lung (the right was not sent) show an extensive tuberculous bronchopneumonia in which caseation had begun. Numerous giant cells were scattered throughout the sections. The pleura

was thickened, and figure 7 shows a unique development of a tubercle in the wall of a branch of the pulmonary vein which had given rise to a polypus-like growth that apparently acted as a ball-valve, and had caused that portion of the vein lying at its left to become widely dilated. At first this vessel was mistaken for a lymph vessel with thickened walls. Its true nature, however, was disclosed by following its course and connections through the series of sections.

Material for the following study was sent me by Doctor Dunham, and the very full history of the case, of which only an abstract is given by Doctor Skavlem, his associate.

Male. White. Age 42 years. Illness extended over three years with a history of frequent colds, persistent and productive cough, night-sweats, and hemoptysis. The first X-ray examination showed cavities in the upper lobe of the right lung, and caseous bronchopneumonia in the upper left lobe. Five months later a cavity appeared in the upper left lobe. The heart was displaced to the right, probably because of adhesions. Sputum positive. Wassermann negative. The necropsy confirmed the X-ray readings.

The case was one of caseous tuberculous bronchopneumonia. Sections showed the right lung to be in a more advanced stage of the disease than the left lung. The thickened pleura proved to be of special interest, for the increase in thickness was due to a different cause in the right lung from what it was throughout the greater part of the left lung.

On the right side dense, fibrous adhesions extended between the lung and the costal pleura. Over the apex of the left lung the adhesions had the same character as on the right side; but over the remaining portion of the lung, in place of there being old, fibrous adhesions, sections showed only the remains of fine, "cobweb" adhesions, with a thin fibrinous exudate on the surface of the lung. The areolar layer was the seat of a generalized tuberculosis in which there were a large number of giant cells, but no definite formation of tubercles (fig. 8). This had led to a thickening of the areolar layer and, consequently, of the entire pleura. The pulmonary structures underlying this particular area of the lung were normal.

Hemorrhage within the *pleura pulmonalis* is not often described as a complication of tuberculosis; it was, however, a feature in the following case, although it is questionable whether it was dependent on the tuberculosis.

Male. Negro. Age 41 years. Admitted to the hospital in which he died, November 28, 1924. Died January 5, 1925. Three months before admission he fell down stairs and in-

jured his back and hips. Following this he had pain in this chest, became weak and could not work. The day before admission he went to another hospital and was told that he had a broken rib, pleurisy and pneumonia on the left side. About a week prior to this he complained of having a cold in his chest, cough and expectoration of bloody sputum.

On admission an X-ray examination showed increased density at the left base, but not as heavy as one would expect in a pneumonia; it was more suggestive of a pleurisy. A second X-ray, taken sixteen days later, showed that the hilum shadow was increased on both sides. Fine soft mottlings were scattered throughout both lungs. These had appeared since the previous X-ray was taken, and probably were due to a widespread tuberculosis. No report of the necropsy accompanied the specimen.

Sections showed the case to be one of caseous tuberculous bronchopneumonia. At one point, some distance below the pleura, there was a small area in which the alveoli were filled with blood (traumatic?). The chief interest centered in the pleura. All the blood vessels within the areolar layer were distended with blood, the capillaries were also distended to their full capacity, here and there rupture of their walls had taken place, and the entire pleura was infiltrated with blood (fig. 9). The alveoli directly beneath the pleura were slightly compressed, but those more deeply situated were normal in size and structure.

The history accompanying the specimen from which the next photomicrograph (fig. 10) was taken, was exceedingly brief and incomplete.

The case was one of advanced tuberculosis, extending over an indefinite number of years, in a man 46 years old. The left lung was the only one sent. There were numerous cavities in the upper lobe. The lower lobe did not contain any cavities, but there was extensive caseation, especially along the *incisura* and about the hilum. Scattered tubercles were present throughout the outer half of the lower lobe.

Figure 10 is a section of a tubercle situated beneath the pleura. Caseation has already begun. Numerous polymorphonuclear leucocytes are situated within the caseation and around its periphery. While the areolar layer of the pleura contains numerous lymphocytes and is increased in thickness, it has not as yet been invaded by the underlying tubercle. There is no evidence of an exudate over the surface of the tubercle, and the mesothelium is intact.

Figure 11 is a section from another part of the same lung as figure 10. Two tubercles appear in the section; one in the angle formed by a septum passing from the pleura into the lung; the other, a short distance beneath the pleura and on the opposite side of the septum. Both tubercles show a more advanced stage than the tubercle in figure 10. The number of polymorphonuclear leucocytes is smaller than in figure 10, the so called epithelioid cells are more numerous, and the amount of

caseation is greater. The areolar layer of the pleura over the first tubercle has become involved in the tuberculous process but, as in the preceding figure, there is no exudate on the surface of the lung, and the mesothelium, except as it has been torn in sectioning, is intact. An interesting point in connection with these two tubercles is the fact that the septum is being invaded by the second tubercle in the same way that the first tubercle has invaded the pleura.

In the following case there is a still further development of the tuberculous process.

Male. Negro. Age 21 years. Admitted to the hospital February 18, 1924. Died, April 6, 1924. Contracted a cough during the summer of 1923, which persisted into the winter. Has had some fever. Until two weeks before admission to the hospital he said he had felt fairly well. Was in bed six days before admission and definitely ill the preceding five days. No report of the necropsy accompanied the specimen.

The entire left lung was received. The upper lobe was free from any tuberculous infection. The lower lobe, however, was the seat of a widespread caseous tuberculous bronchopneumonia, which was especially well marked about the hilum. This was a good example of lower-lobe tuberculosis.

In figure 12, which is a section from the lateral surface of the lower lobe, caseation has advanced to a point just short of liquefaction. Within the caseation numerous pyknotic nuclei are seen; around its periphery polymorphonuclear leucocytes, a few lymphocytes, and the so called epithelioid cells are present. There is also, especially around the lower border of the caseation, the beginning formation of a capsule made up of fibrils of reticulum and a few collagenous fibres. The areolar layer of the pleura over the caseous area is beginning to be involved in the tuberculous process. Situated beneath this tubercle, and separated from it by a row of compressed and partially obliterated alveoli, there is a smaller, somewhat younger tubercle, which is also caseous in its centre. Eventually these two tubercles might have fused and given rise to a cavity of considerable size; or they might have formed two small cavities, separated from each other by a common wall of reticulum and collagenous fibres.

When tubercles similar to those in figure 12 liquefy, a cavity results. An example of such a cavity was found in the following case:

Male. White. Age 37 years. Ill for two years. Had lost over fifty pounds in weight. Had tuberculosis of the left knee for which amputation was performed twenty months before

he died. Sputum at that time was positive and the X-ray showed extensive tuberculosis of both lungs.

Figure 13 is a section taken from the lateral surface of the lower left lobe, and is an example of a cavity situated immediately beneath the pleura. This type of a cavity does not, as a rule, lead to perforation of the pleura. Extensive destruction of the pulmonary tissue may take place and a cavity of large size may result, but the pleura remains intact.

Had the individual from whom this section was obtained lived longer, in all probability the four cavities, which appear in the section, would have given rise to one large cavity, and the thrombosed, canalized artery, which is seen in the lower part of the large cavity, might have formed a trabecula passing through it; or the walls of the artery might have given way and a hemorrhage of greater or less severity ensued. The probabilities are, however, that it would have become thrombosed throughout its entire length and have undergone tuberculous necrobiosis.

Why the destructive process is arrested at the areolar layer of the pleura in these cases is a question which needs further study.

Tuberculosis of the pleura is not a primary disease: it is secondary to tuberculosis elsewhere in the body, usually in the lungs or in the bronchial lymph nodes. If in the latter, the primary focus was undoubtedly in the lung, though it may have been so small as to escape detection.

There are two possible routes by which bacilli may reach the submesothelial layer; the one from without the lung, the other from within the lung. In either case they are conveyed by phagocytes of the macrophage type, of which the exact name and source are yet to be determined.

In the case of the areolar layer, tubercles may be of lymphogenous origin (figs. 5 and 6) or of hematogenous origin (fig. 7); or there may be an extension from a tuberculous process in the underlying pulmonary structures.

When tubercles situated just beneath the pleura liquefy and give rise to a cavity (fig. 13) of greater or less extent, the pleura does not, as a rule, take part in the tuberculous process.

As a possible explanation the following thesis is advanced: The collagenous fibres and the layer of elastic fibres are definitely resistant to

the extension of the tuberculous process. The blood and lymph vessels within the pleura provide a means by which the products of the tuberculous inflammation are quickly removed.

When, however, the entire thickness of the pleura becomes involved, adhesions are soon formed between the *pleura pulmonalis* and the *pleura costalis*, and additional support is provided by the thoracic wall. This opens up another phase of the subject, which will be considered in a future publication.

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DESCRIPTION OF FIGURES

All photomicrographs are from untouched negatives

FIG. 1. NORMAL HUMAN PLEURA

Elastic fibres stained. The dark lines which appear here and there on the surface of the pleura are the remains of the mesothelium, which had been partially rubbed off at the time the lung was removed. *A*: Submesothelial layer. B: The elastic layer. C: The areolar layer. D: Elastic fibres which belong to the pulmonary alveoli. × 50.

FIGS. 2, 3 AND 4. SUCCESSIVE SECTIONS THROUGH A SUBMESOTHELIAL TUBERCLE

2: Haematoxylin and eosin staining. *3*: Reticulum stained; the heavily stained fibres are *collagenous* fibres. *4*: Elastic fibres stained. (See AMER. REV. TUBERC., 1923, vii, 152.) × 50.

FIG. 5. TUBERCULOSIS OF THE AREOLAR LAYER AND UNDERLYING PULMONARY TISSUE

A young tubercle developing in the wall of a lymph vessel. Exudate on the surface of the pleura. Blood vessels dilated. Pleura much increased in thickness. × 50.

FIG. 6. FROM THE SAME LUNG AS FIGURE 5

A more highly developed tubercle situated at the point where lymph vessels divide. Areolar layer oedematous. × 50.

FIG. 7. A POLYPUS-LIKE GROWTH OF A TUBERCLE INTO THE LUMEN OF A PULMONARY VEIN, SITUATED IN THE AREOLAR LAYER OF THE PLEURA, THAT CAUSED THAT PORTION OF THE VEIN AT ITS LEFT TO BE WIDELY DILATED

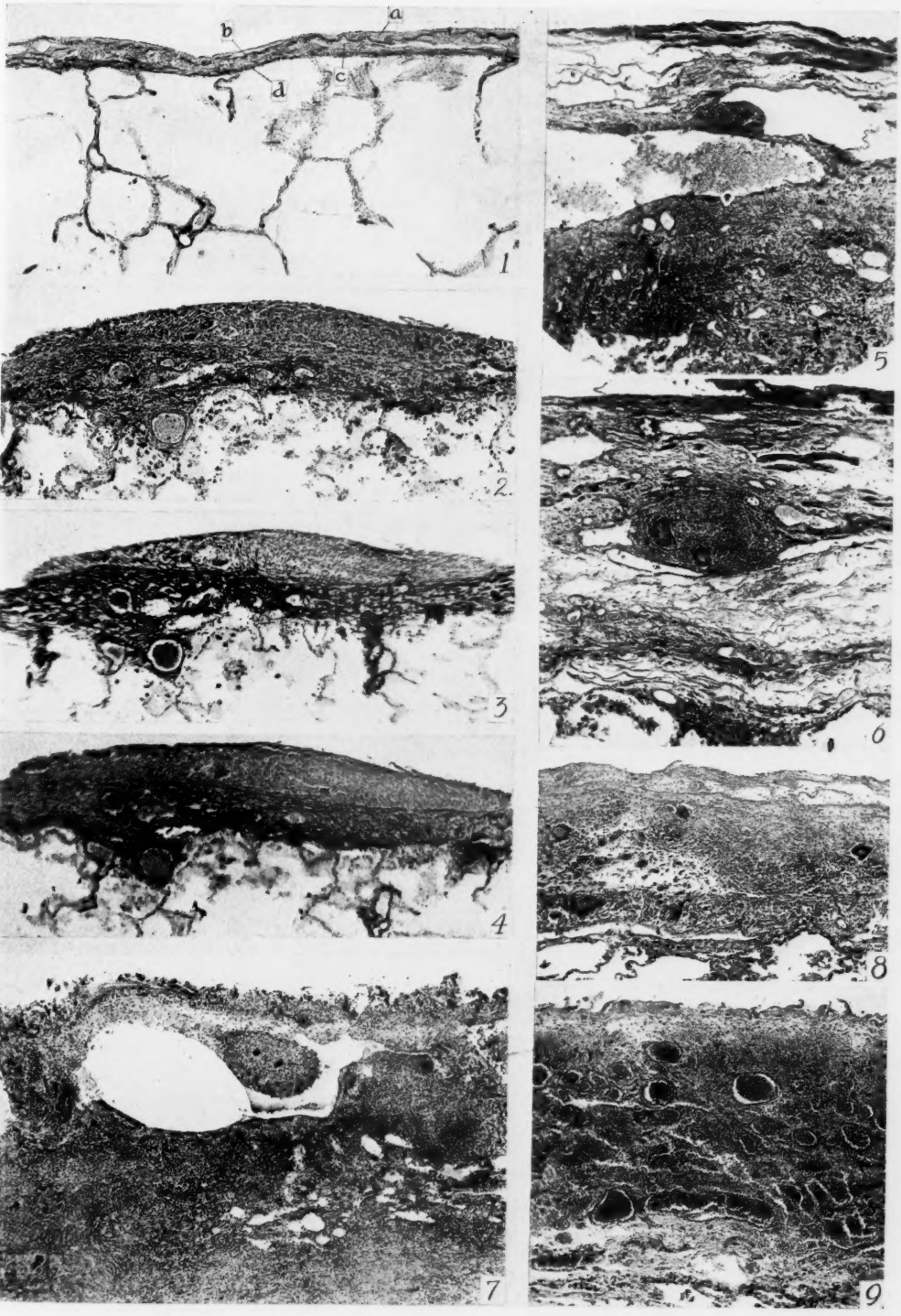
Underlying pulmonary structures tuberculous. × 25

FIG. 8. GENERAL TUBERCULOSIS OF THE PLEURA

Note the scattered giant cells without the formation of distinct tubercles. × 50

FIG. 9. HEMORRHAGE WITHIN THE PLEURA

The ruptured walls of the dilated capillaries can be made out with the aid of a reading glass. × 50.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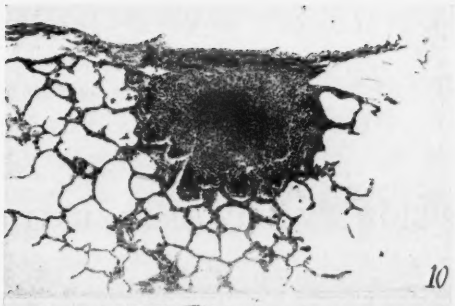
DESCRIPTION OF FIGURES

FIG. 10. A YOUNG TUBERCLE BEGINNING TO INVADE THE PLEURA. × 25

FIG. 11. TWO TUBERCLES MORE ADVANCED IN DEVELOPMENT THAN IN FIGURE 10
One has invaded the pleura; the other a septum. × 25

FIG. 12. TWO TUBERCLES IN WHICH CASEATION HAS ADVANCED TO A POINT SHORT OF LIQUEFACTION
The larger of the two has invaded the pleura as far as the elastic layer. × 25

FIG. 13. CAVITIES FORMED BY THE LIQUEFACTION OF SUCH TUBERCLES AS ARE SHOWN IN FIGURE 12
Note the thrombosed and canalized artery in the larger cavity. × 7.5



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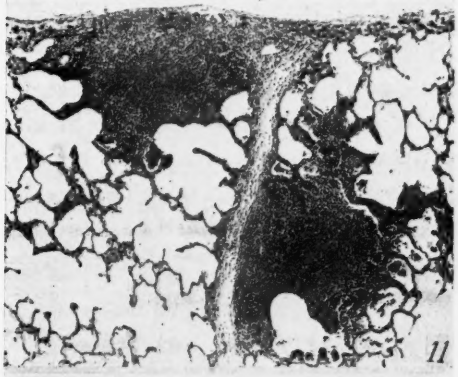

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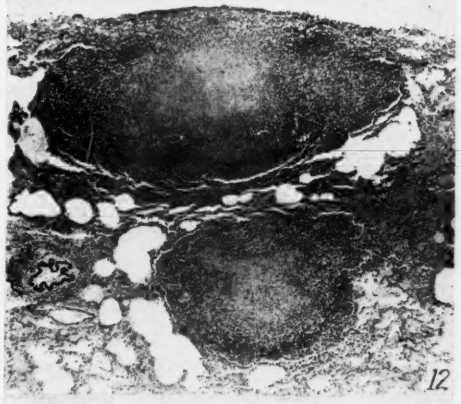

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13

THE TENSION THEORY OF PLEURITIC PAIN[1]

H. A. BRAY[2]

There are two important theories regarding the mechanism of pleuritic pain. One, the more generally accepted, ascribes pleuritic pain to increased friction due to roughening of the pleural surfaces by the inflammatory exudate; the other, advocated by Sir James MacKenzie, attributes the pain to tonic contraction of the hyperalgesic intercostal muscles. Both theories apparently are open to objection. My observations indicate that tension of the inflamed parietal pleura best explains the characteristic inspiratory nature of pleuritic pain.

The Friction Theory: This theory has gained favor chiefly because (1) the area of friction sounds and the area of pain often correspond; (2) the intensity of the friction sounds and the severity of the pain apparently are closely related, inasmuch as both are increased on deep inspiration; (3) the friction sounds are loudest at the base of the chest, the site of the severest pain; (4) the pain is alleviated by mechanical immobilization of the ribs, which is said to reduce friction; and (5) the pain is relieved when the friction is eliminated, as by an effusion or a pneumothorax.

It is indisputable that the area of friction and the area of pain may correspond, but it is equally true that they may not. Moreover, friction sounds often persist after the pain has subsided, or may be detected in patients who at no time have suffered from pleuritic pain. Conversely, sharp pain is often present without detectable friction. Failure to detect friction does not necessarily exclude its presence, for it is conceivable that friction sounds may be present, but of such slight intensity as to escape detection by our present methods of examination. However, if inaudible friction can cause severe pain, then there is not the supposedly intimate relation between the intensity of the friction sound and the sharpness of the pain.

[1] This study was made at the New York State Hospital for Incipient Pulmonary Tuberculosis at Ray Brook, New York, and at the Bellevue Hospital, Cornell Medical Division, New York City. Read at the forty-second annual meeting of the American Climatological and Clinical Association, Washington, D. C., May 6, 1925.

[2] Ray Brook, New York.

If the pain were caused by friction, the pain, like friction, should be of equal intensity during both phases of respiration, because the glide of the inflamed pleural surfaces and, therefore, the amount of friction produced are the same on inspiration and expiration. In reality, the pain is usually absent on expiration and severe on inspiration, despite the fact that friction sounds may, at times, be of the same intensity during both phases of respiration. This lack of correlation between pain and friction is further shown when the breath is held at the height of inspiration, the period of maximum pain. If the pain were caused by friction, it should cease when the breath is held and friction suspended. This does not occur, for the maximum severity of pain is maintained until expiration begins.

The relief from pain after mechanical fixation of the ribs is attributed to lessened friction. However, a fluoroscopic study shows that immobilization of one side of the chest by strapping causes a decided increase in the excursion of the corresponding diaphragmatic leaf. Thus, if on costal inspiration the excursion of the diaphragm be one inch, it will be increased to approximately two inches when the ribs are immobilized. In other words, this procedure alters the direction of, but does not materially lessen the glide of the pleural surfaces. Hence, friction is not reduced, and the consequent relief from pain by mechanical immobilization of the ribs cannot reasonably be attributed to lessened friction.

The separation of the pleural surfaces and the cessation of friction by the advent of an effusion or pneumothorax are brought forward in support of the friction theory. As a matter of fact, they argue against it, a point which will be discussed later.

The friction theory is based principally on the supposition that the pleural surfaces are roughened by an inflammatory exudate. At necropsy, I have often failed to find palpable roughening of the inflamed parietal pleura, although the patient had suffered from severe pleuritic pain. Conversely, the pleural surfaces at times have been found acutely inflamed and decidedly roughened, yet the clinical reports failed to show a complaint of pleuritic pain.

After all, the coexistence of pain and friction sounds does not warrant the conclusion that friction is an important causal factor of pleuritic pain.

The Muscular Theory: MacKenzie attributed the pain of pleurisy to hyperalgesia and tonic contraction of the intercostal muscles. Hyper-

algesia of the intercostal muscles cannot be satisfactorily demonstrated, owing to their inaccessible location. Nevertheless, MacKenzie's assumption that they become hyperalgesic in pleurisy is probably correct, because this condition occasionally can be demonstrated in the more accessible thoracic muscles, including the pectorals. I have, however, noted that forcible contraction of these accessible hyperalgesic muscles by passive resistance fails to arouse pain. Granted that pleurisy causes hyperalgesia of the intercostal muscles, it is still open to question whether their contraction evokes the violent inspiratory pain of pleurisy.

MacKenzie (1) believed that the pleura is not supplied with sensory nerves and is therefore incapable of evoking pain. Although recorded observations justify the conclusion that the visceral layer of the pleura is insensitive, the following studies demonstrate that the parietal layer is sensitive to pain. Dogiel (2) has demonstrated sensory end-bulbs in the human parietal pleura, and Dr. M. J. King, my former associate, found them in the parietal pleura of the rat, calf, rabbit and guinea pig. The presence of these nerve end-bulbs presupposes a pain sense in this structure. Capps (3) found that the mechanical irritation of the inner surface of the diseased parietal pleura nearly always produced pain, "sharp and localized over the area irritated." In operations under local anaesthesia Sewell (4) noted that manipulation of the healthy nonanaesthetized parietal pleura evoked exquisite pain. Furthermore, the occurrence of sharp pain in thoracentesis, when the needle punctures the outer layer of the pleura, has been noted repeatedly. These observations apparently disprove the idea that the parietal pleura is not sensitive to pain.

The Tension Theory is so termed because it attributes pleuritic pain to tension or stretching of the inflamed parietal pleura.

It is well known that in acute pleurisy at the summit of the chest local pain is frequently absent. When present, it is rarely severe, usually dull and aching in character, fairly constant, and not modified by respiration. This pain is due to inflammatory irritation of the sensory nerves of the costal pleura. In acute pleurisy at the base of the chest the dull, aching pain may likewise be present or absent, but in either event there is usually agonizing pain on inspiration and partial or complete relief of the pain on expiration. This conspicuous difference between the pain at the base and that at the summit of the chest on inspiration is not attributable to any appreciable difference in the acute-

ness of the inflammation in these areas. I have noted at necropsy that the costal pleura was as acutely inflamed at the summit as at the base, yet the agonizing inspiratory pain had been confined to the base of the chest. Consequently, there is reason to assume that some factor, acting on the inflamed pleura at the base, but not at the summit of the chest, is directly responsible for the inspiratory pain. This factor, in my opinion, is tension.

Miller (5) states; "Wherever elastic fibres are found, one is justified in ascribing to them the function of permitting extension and relaxation." The parietal pleura is abundantly supplied with elastic fibres, and is firmly attached to the inner surface of the ribs. Therefore, separation of the ribs increases the tension of the parietal pleura. On inspiration, the ribs separate appreciably at the base, but not at the summit of the chest, a point noted by Austin Flint, Sr., (6) and others. Anteriorly, at the base of the chest and in the axilla, their separation is greater than posteriorly. Hence, in pleurisy, tension of the diseased pleura is heightened at the base of the chest, particularly anteriorly and in the lower axilla, areas which represent the site of the inspiratory pain and the usual points of its greatest severity. As the tension gradually heightens during inspiration, the severity of the pain likewise progressively increases, until at the end of inspiration the maximum of tension and pain is reached. If the breath is held at this point, the maximum pain is sustained until tension is relieved by expiration. As the tension lessens during expiration, the pain is gradually relieved, and often it disappears completely toward the end of the expiratory act. At the summit of the chest the absence of pleural tension explains the absence of the inspiratory pain in this region.

The posture of the patient in pleurisy may be interpreted as Nature's effort to relieve pain. In costal pleurisy the patient bends his body toward the affected side. This position approximates the ribs and lessens tension of the costal pleura. Tonic contraction of the intercostal muscles and shallow respirations restrain the excursion of the approximated ribs and keep the inflamed pleura relaxed. Conversely, if the patient bends his body toward the healthy side, tension of the diseased pleura is increased by separation of the ribs and the pain is immediately aggravated.

DuBois (7) observed that one of his patients suffering from pleurisy was lying on his healthy side, and further noted that the bed sagged greatly in the middle. I have confirmed this observation on several

occasions. The sag bends the body so that the under ribs are separated and the ribs on the diseased side are approximated, thereby relieving tension of the diseased pleura.

There may be no characteristic posture with diaphragmatic pleurisy; however, in certain instances the posture resembles that of acute peritonitis. The patient lies on his back, quite still, with the knees flexed, the abdominal muscles rigid, and the diaphragm partly or completely immobilized. The respirations are purely costal, and the patient makes no attempt to bend his body laterally. Here Nature's efforts are directed toward immobilization of the inflamed diaphragmatic pleura. When both the diaphragmatic and costal pleurae are inflamed, posture is ineffectual in alleviating pain, for any attempt to lessen tension of the costal pleura by posture would proportionately disturb the diaphragmatic pleura by increasing diaphragmatic excursion, and vice versa.

In pneumothorax, the pleural surfaces are widely separated, and hence friction cannot be a factor in the production of the excruciating pain associated with this condition. The rapid distention of the pleural cavity by air causes a sudden and marked tension of the diseased parietal pleura. This is probably responsible for the severe pain, since it is most marked at the base of the chest, where the separation of the ribs is widest and tension greatest. The degree of pleural tension and the severity of the pain apparently are closely related. Thus, in pneumothorax under pressure the pain is often promptly alleviated by thoracentesis, which relieves intrapleural pressure, approximates the ribs and lessens pleural tension. The adhesions encountered in pneumothorax tug on the diseased costal pleura and cause pain. When they are confined to the summit of the chest, the severest pain may occur in this region.

The adherents of the friction theory believe that cessation of pleuritic pain with the advent of an effusion is due to the elimination of friction. This view is questionable, because in artificial pneumothorax pleuritic pain likewise subsides with an effusion. Here elimination of friction is obviously not a factor in the cessation of the pain. The effusion denotes that the inflammation has entered the exudative stage, and one may possibly attribute the cessation of pain to the consequent relief of pressure on the nerve terminals of the pleura. Moreover, in ordinary pleurisy with effusion, the fluid, by immobilizing the ribs, prevents tension of the pleura and hence contributes to the relief of the pain. When the movement of the ribs is not sufficiently restrained, as in the

case of small effusions, the pain may persist. Large effusions, which bulge the intercostal spaces and increase the tension of the costal pleura, should cause pain, according to the tension theory. Such effusions incite pain when the pleura is acutely inflamed. Conversely, when the inflammation is chronic, as in tuberculous effusions, there may be no pain. In other words, when the effusion causes tension of the pleura, the presence or absence of pain is determined by the state of the inflammation.

The duration of the pain in pleurisy is limited. For example, in the acute pleurisy complicating lobar pneumonia the pain often ceases after the third or fourth day. In some manner, the inflammation fails to incite pain after a certain interval. When the inflammation is of long standing, as in chronic empyema, the pleura may even become temporarily insensitive. MacKenzie encountered such instances of insensitiveness of the pleura, and apparently was led thereby to the erroneous conclusion that the pleura is not supplied with sensory nerves and is therefore incapable of arousing pain. He stated (8), "In a great number of cases, when the ribs have been resected, I have repeatedly explored the pleural cavity for any evidence of sensation, and I could employ no form of stimulation capable of producing pain."

The comparative sensitiveness of the costal pleura in its different parts has not been established. If future investigations should show that the structure is more sensitive in the lower than in the upper portion, and that there are zones of varying degrees of sensitiveness in the lower portion, it would be an important factor in further explaining the localization and points of distribution of the pain at the base of the chest. There are other interesting features about pleuritic pain, including the occasional absence of pain in acute pleurisy, hyperaesthesia of the skin, hyperalgesia of the thoracic muscles, and the paths of distribution of the referred pains, which require further study.

This study indicates that neither the friction theory nor the muscular theory satisfactorily explains the characteristic inspiratory pain of pleurisy. This, the cardinal feature of the pain, I believe is due to tension of the inflamed parietal pleura.

I wish to thank my former associates at Ray Brook, Dr. Bruce Whyte and Dr. Merrill J. King for their kind assistance and valuable suggestions in this study.

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A NEW PALLIATIVE METHOD FOR THE RELIEF OF SEVERE PLEURITIC PAIN IN PULMONARY TUBERCULOSIS[1]

GEORGE I. SWETLOW[2]

This paper presents five cases as a preliminary report of an apparently new method in the controlling of severe thoracic pain caused by pleuritic involvement. A careful search of the literature for any reference to this method has revealed no record of its previous use.

Although it is quite true that severe, agonizing thoracic pain is not the common observation in pulmonary tuberculosis, there are, nevertheless, many cases in which the distress is so severe that it responds to no treatment. The method to be described, although just begun, indicates a means for the amelioration of the intense pain.

The injection of alcohol into peripheral nerves for the relief of pain is well known. Its success in the treatment of various forms of neuralgia such as trifacial neuralgia and sciatica, has been clearly demonstrated. The method to be described attempts to inject alcohol into or very near the dorsal root ganglia, for the purpose of producing an axonal degeneration.

The upper six intercostal nerves supply the upper parietal costal pleura. The lower six send their intercostal fibres to the lower parietal costal pleura, as well as to the parietal pleura of the diaphragm. The centre of the diaphragm is supplied by the phrenic nerve, which comes mainly from the fourth cervical nerve and also from the third and fifth cervical nerves of the cervical plexus. The phrenic nerve is not only motor to the diaphragmatic mucles, but also carries afferent sensory impulses from the parietal pleura to the cervical plexus. Disease, therefore, in the costal parietal pleura will transfer its effects in the form of pain to the chest wall. The site of reference will depend upon the intercostal nerves impinged upon. However, irritation of the

[1] From the Tuberculosis Service, Montefiore Hospital for Chronic Diseases, Dr. M. Fishberg, Chief of Service.

[2] Resident, Tuberculosis Service, Montefiore Hospital for Chronic Diseases, New York City.

parietal pleura over the central portion of the diaphragm will refer the pain to the neck, which is the area supplied by the third, fourth and possibly fifth cervical nerves.

Ranson, in his original researches on peripheral nerves, showed that a sensory peripheral nerve is made up of both myelinated as well as unmyelinated fibres, that the unmyelinated fibres are in great preponderance, and that the unmyelinated fibres carry the pain-bearing influences (*protopathic*). Since these fibres are peripheral arms of cells situated in the dorsal ganglia it is quite evident, theoretically, that the destruction of these peripheral arms or ganglia would destroy the pathway of the afferent stimuli, and thus prevent the influx of pain stimuli to the consciousness. Since the nerve itself not only hugs the under margin of the rib but also runs along with the intercostal artery and vein, it was considered inadvisable to attempt to inject the nerve itself along the thoracic course. The more accessible structures however are the dorsal ganglia or dorsal root fibres entering the ganglia. The ganglia are found in the intervertebral spaces. The thoracic nerves, immediately upon emerging from the intervertebral foramina, lie midway between the transverse processes of the two adjacent vertebra, and run obliquely across the intercostal spaces toward the angles of the ribs above them to enter the subcostal grooves. As the nerves are coursing from the intervertebral foramina to the angles of the ribs, they are found lying on thin aponeuroses known as the internal intercostal fasciae. These fasciae extend to the tubercles of the ribs. The aponeuroses are the only layers separating the nerves in this location from the pleural cavity. This anatomical fact must be borne in mind during the injection.

Technique: After having chosen the intercostal nerves to be injected, the following procedure is executed: The patient is placed on the side opposite to the one to be injected. The knees and hips are flexed on the abdomen and the head approximated to the lower extremities. This widens the intercostal spaces. Taking the twelfth rib as a landmark, the intercostal nerves to be injected are carefully palpated. A line running through the midline of the vertebral spinous processes is traced on the skin. At a point 4 cm. from this line and over the ribs which are above the spaces to be injected, wheals of 2 per cent novocain are raised. The wheals act both as a local anesthetic and as a landmark. An 8-cm. needle is inserted through the wheal. The needle is directed perpendicular to the posterior surface of the rib just above the

space to be injected. As soon as the needle impinges upon the rib, it is slightly withdrawn in order to change its direction. The direction of the needle is changed so that its shaft points 45° downward, inward and forward toward the lower border of the rib. From the lower border of the rib the point is advanced 2 cm. further. The point finds itself between the internal and external intercostal muscles. The needle is then attached to a water manometer, to determine whether it is in the pleural cavity. An absence of extensive oscillations indicates that the needle is not in the pleural cavity. This precaution is taken to obviate the injection of alcohol into the pleural cavity. Three to 5 cc. of novocain is then introduced through the several needles. After waiting a few minutes the areas are tested for the presence of the previous hyperalgesia. With the disappearance or diminution of the hyperalgesia, we have concrete evidence that the point of the needle is in the correct spot. Two to 3 cc. of 80 per cent alcohol (warm) is injected through the same needle. Half of the fluid is injected without moving the needle, while the rest is distributed with a little to-and-fro movement as the point is being withdrawn. The alcohol thus deposited produces an axonal degeneration of the thoracic nerve very near to its cell body in the dorsal ganglia. Some of the alcohol may even spread along, to reach the intervertebral foramen and exert its action upon the ganglion itself. The technique described is for the injection of the thoracic intercostal nerves.

The following technique to be described is for the approach to the second, third and fourth cervical nerves, to control shoulder pain incident to diaphragmatic irritation. The patient is placed in the lateral recumbent position. Wheals are raised opposite the spinous processes of the fourth, fifth and sixth cervical spines, on a line 3 cm. distant from and parallel to the midline of the cervical spinous processes. An 8-cm. needle is introduced parallel to this line running through the spinous processes, and advanced up to the point where the vertebral arch is impinged upon. Here the point is slightly withdrawn, and inclined outward, so as to have the needle pass the arch of the vertebra at a tangent. As soon as the needle loses contact with the bone the point is introduced for 1 cm. deeper. This same procedure is carried out opposite the cervical spines of the fourth, fifth and sixth cervical roots. Novocain, 2 per cent, is introduced, and the skin area tested for the previous zone of hyperalgesia. With its diminution or absence, we have good indication that the correct nerves are being infiltrated. Only then are 2 to 3 cc. of 80 per cent alcohol injected through the needles.

The following is a summary of the five cases treated by this method:

Case 1: W. S. Male. Age 57 years. For five weeks before the beginning of the treatment, the patient complained of a persistent, severe, sharp pain over the right posterior thoracic and axillary regions. The distress was greatly increased on movement or coughing. The pain was especially severe during the night, preventing sleep. Physical examination showed the patient to be suffering from a far-advanced, bilateral, chronic pulmonary tuberculosis, fibrocaseous in nature. On auscultation over the area complained of, very feeble breathing was heard. The X-ray showed a far-advanced, bilateral lesion, with marked adhesions to the ribs and diaphragm on the right side. A zone of hyperalgesia was elicited over the sixth and seventh intercostal spaces. Deep pressure over these spaces elicited great tenderness. After the injection of this nerve the following summarizes the course: (1) For three days after the treatment, the patient complained of severe pain at the site of the alcohol injection. Following this his pain completely disappeared over the area of his previous complaints. (2) The area of hyperalgesia over these spaces disappeared. (3) It is now a total of 106 days since he was treated and relieved.

Case 2: A. S. Female. Age 19 years. For four months previous to the injection the patient complained bitterly of severe, sharp, sticking pains in her left middorsal region and axilla. These pains were especially severe during the night. Coughing greatly exaggerated the pain. Physical examination showed a far-advanced, bilateral, fibrocaseous lesion. Over the left middorsal area, signs of a thickened pleura were elicited. The X-ray examination confirmed this finding. A zone of hyperalgesia was elicited over the left side, that extended over the dermatomic segments supplied by the sixth, seventh and eighth intercostal nerves. On pressing firmly over these nerves great tenderness was elicited. The sixth and eighth intercostal nerves were injected. The following summarizes the patient's course after the injection: (1) For two days she complained of pain over the local area injected with the alcohol. There was, however, very little pain over the back and axilla. (2) The zone of hyperalgesia had disappeared over the skin areas supplied by the sixth, seventh and eighth intercostal nerves. (3) Deep pressure over the intercostal nerves produced no pain. (4) She definitely asserted that the pain in the axilla had disappeared. (5) After a period of 32 days' relief she died from the ravages of the disease.

Case 3: H. B. Male. Age 45 years. For six weeks before the injection the patient complained of intense, agonizing, sticking and tearing pain over the right scapular region. The pain was increased on the slightest movement.

Lying on his back induced such agonizing pains as to prevent all sleep. In fact, he walked the wards several nights because of his inability to recline in bed. Physical examination showed a far-advanced, bilateral, pulmonary tuberculosis, with a friction rub over the right upper chest. There was a zone of hyperalgesia that extended from the first to the sixth thoracic nerves inclusive. Marked pain was elicited on deep palpation over these nerves. The following summarizes the patient's course after the injection: (1) On the day after the injection, all pain in the back disappeared. (2) He was able for the first time in several weeks to recline on his back. In fact, all pain in the area had disappeared. The zone of hyperalgesia had disappeared as well as the tenderness elicited on deep intercostal palpation. It is now a total of 70 days since he was treated and relieved.

Case 4: L. H. Male. Age 40 years. For four months before the injection, the patient complained of a sticking, tearing pain, which began in the midposterior chest on the right side and radiated anteriorly. The pain was constant, being present both day and night. Often the distress was so severe as to prevent sleep. Coughing greatly aggravated the pain. Strapping of the chest gave the patient only slight relief. Physical examination showed a bilateral pulmonary lesion with cavity formation. A zone of hyperalgesia was elicited over the seventh, eighth, ninth and tenth intercostal nerves. Marked pain was elicited on deep palpation over these nerves. The seventh, ninth and eleventh roots were injected. The following summarizes the patient's course after the injection: (1) One day after injection, the thoracic pain disappeared completely. (2) He was able for the first time in months to recline on his back and sleep comfortably. (3) He complains at times, however, of a slight burning paresthesia over the area injected. (4) He definitely asserts that he is much relieved. (5) It is now a total of 62 days that he was treated and relieved.

Case 5: M. S. Male. Age 48 years. For four weeks before the beginning of the treatment, the patient complained of a persistent, severe, sharp pain over the right lower chest. Frequently the pain was constricting in character. The distress was greatly aggravated on movement or coughing. The pain was especially severe during the night, preventing sleep. A physical examination confirmed by X-ray findings showed that he was suffering from an advanced, bilateral, chronic pulmonary tuberculosis, fibrocaseous in nature. A zone of hyperalgesia was elicited over the seventh, eighth and ninth intercostal spaces. Deep pressure over these spaces elicited great tenderness. After the injection of this nerve the following summarizes the course: (1) The pain has disappeared, so that the patient is able to sleep during the night. (2) Coughing and moving about initiate no pain. (3) The zone of hyperalgesia has been greatly

diminished. (4) Deep pressure over the intercostal spaces produces no pain. (5) It is now a period of 38 days that he has been treated and relieved.

SUMMARY

1. Five cases of severe chest pain, due to pleuritic involvement, were treated by a new method and are presented as a preliminary report.

2. The results are so encouraging that further work along these lines is indicated.

I wish to thank Dr. M. Fishberg for his kind encouragement in this work.

THE TREATMENT OF TUBERCULOUS PERITONITIS BY OXYGEN INFLATIONS[1]

E. W. HAYES[2]

It is my purpose to call attention to a method of treatment of certain forms of tuberculosis which has been very efficient in the hands of the few who have used it, a method which I believe at this time merits wider application. Primarily this treatment consists of the inflation of certain closed cavities with oxygen. In this paper I will deal largely with tuberculous conditions within the abdomen.

The technique we have used in introducing the oxygen has been the same as that used by us in artificial pneumothorax. The same asepsis is employed as in opening the abdomen. The air-chamber in the pneumothorax outfit is filled with oxygen just before the operation. The abdominal wall is carefully infiltrated with one per cent solution of novocain containing 4 minims of adrenalin to the ounce. Precaution is taken to thoroughly infiltrate the skin and the peritoneum. In those cases in which it is not necessary to aspirate we use the Salimen catheter to introduce the oxygen into the abdominal cavity. In the ascitic cases we use a specially made trocar, gauge 11. After the fluid is withdrawn the oxygen is introduced through the trocar. The favorite site for introducing the needle is about one and a half inches below and one and a half inches to the left of the umbilicus. The amount of oxygen depends to some extent on the case. In our experience, from 300 to 800 cc. seems to be the optimum amount. In cases relatively free from adhesions, liver dulness usually disappears, and the fluid in the manometer begins to oscillate corresponding to the movement of the diaphragm when 200 to 300 cc. of oxygen are introduced. The length of interval between refills varies from a few days to two or three weeks or longer, and is determined by the condition of the patient.

It is generally admitted that about half of the cases of tuberculous peritonitis will recover under the usual regimen for tuberculosis, while a

[1] Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

[2] Monrovia, California.

certain proportion of the other half will recover only if they receive surgical intervention in addition. The main surgical procedure has been to open the abdomen, evacuate any fluid, expose the abdominal contents to the air for varying lengths of time, and close without drainage. In the type of tuberculous peritonitis in which the disease was more or less localized, as in the pelvis, appendix or a limited portion of the bowels, removal of these areas has been followed by good results. A few surgeons have used various antiseptic or cleansing solutions in the tuberculous abdomen, while a few others have exposed the abdominal contents to the direct rays of the sun for periods up to forty-five minutes. With neither of these latter procedures have the results been very encouraging. In still other operative cases oxygen in a continuous stream has been used for irrigating the abdominal cavity after the ascitic fluid had been drained, or the abdomen has been inflated with air or oxygen immediately following operation. The results in both of these procedures have been beneficial.

Bainbridge (1) cites the fact that in 1761 Maniere and Gimbernat used injections of sterilized air in the treatment of hydrocele, and that Marcane and DeMarquay in 1865 announced the cure of senile gangrene by oxygen injection, and that in 1799 Beddoes employed oxygen for the cure of certain ulcers.

In 1909 Bainbridge himself reported the cure of tuberculous ulceration of the intestine, tuberculous peritonitis and other infectious processes by oxygen injection.

Meeker (2) in 1912, Davis (3) in 1913, Stein (4) in 1922, and Mattick (5) in 1924 all reported very excellent results secured by the inflation of the abdomen with oxygen in cases of exudative tuberculous peritonitis. In September, 1912, Goodwin (6) stated, "In the past four years I injected oxygen into the peritoneal cavity after abdominal operations and must say the results in selected cases have been very encouraging." He stated that since he had used this treatment in the ascitic form of tuberculous peritonitis he had never seen the condition return.

In September, 1920, Rost (7), working in India, reported the treatment of 8 cases of tuberculosis of closed cavities by inflation of oxygen with very satisfactory results. There were 4 cases of psoas abscess, 3 of tuberculosis of the knee joint, and one of exudative tuberculous peritonitis. In December, 1921, Rost reported 24 additional cases of tuberculosis in closed cavities treated by oxygen inflation with extremely good results.

In December, 1921, Sargo and Fritz (8), of Berlin, reported very suc-

cessful results in their practice and that of some of their colleagues in the treatment of exudative types of tuberculous peritonitis by aspiration followed by oxygen inflation.

In 1923 Bainbridge (9) stated, "In 250 laparotomies I have used oxygen in the peritoneal cavity with uniformly good results."

In May, 1924, Purves and Bilcliffe (10) reported the results of their experience in the treatment of 21 cases of tuberculosis in closed cavities. Their patients were not selected except that no cases with sinuses were treated. The patients were allowed to return to their homes to convalesce, and this in many instances meant unfavorable surroundings. Of the 21 cases 10 recovered, 2 improved, 6 failed, 2 died and 1 could not be traced.

Weil and Loileseur (11) in 1921 reported the treatment of 6 cases of tuberculous peritonitis with ascites by the injection of filtered air into the abdomen, after the fluid was withdrawn. Three cases were cured and 3 failed.

Gilbert (12) in 1924 reported excellent results in 2 cases of exudative peritonitis treated by injections of air.

Laney (13) in 1924 reported marked alleviation of symptoms as well as general improvement in a case of intestinal ulceration complicating far-advanced pulmonary tuberculosis, treated by oxygen inflation of the abdomen.

In our clinic and in our work at the Los Angeles General Hospital we have used oxygen inflation therapeutically since July, 1923. It has been used principally in the treatment of tuberculous peritonitis and enteritis. In addition, we have used it in 2 cases of tuberculous abscess. At this time I am reporting 3 cases of tuberculous peritonitis in detail, inasmuch as these represent the three somewhat distinct forms of tuberculous peritonitis encountered.

Case 1: Girl, fifteen years old, had previously been under treatment for glandular tuberculosis. She was seized with sudden abdominal pain, temperature 103°, pain somewhat localized around McBurney's point. The abdomen was opened, and found to be studded throughout with miliary tubercles. There was no fluid or adhesions. Following the operation the patient was placed under the general care for tuberculosis, with the application of the Alpine rays to the abdomen. For two weeks there was a general improvement. At the end of this time symptoms again became more marked, the pain and discomfort increased, and the temperature rose to 104°. There were a gradual loss of weight and inability to take food, and

the bowels were markedly constipated. This condition continued for six weeks. The patient finally reached the stage where she was unable to retain anything, even water, in her stomach, and it was impossible to secure an evacuation of the bowels. By this time she had lost 50 pounds. The abdomen was only markedly tender, pain was not a prominent symptom, and there was no evidence of fluid and no marked distention. Three hundred and fifty cubic centimetres of oxygen were injected through the abdominal wall into the abdominal cavity. There was no further vomiting. The bowels afterward moved either naturally or with a mild laxative, and within forty-eight hours her appetite, which had been gone for weeks, returned. Her temperature on the twenty-first day after the operation had decreased to 99°, with a corresponding reduction in the pulse-rate. In two months she was entirely free from symptoms and had regained most of her weight. She has been kept under careful supervision but was allowed to return to school in September, 1924, and has continued well since. The injections were as follows:

July 25, 1923, 350 cc. oxygen, beginning reading 0, end reading positive 3 cm. water pressure.
August 7, 1923, 400 cc. oxygen, beginning reading positive 1, end reading positive 3 cm. water pressure.
August 21, 1923, 200 cc. oxygen, beginning reading 0, end reading positive 3 cm. water pressure.
September 4, 1923, 250 cc. oxygen, beginning reading 0, end reading positive 4 cm. water pressure.

Case 2: A boy, eighteen years old, referred by Dr. Clarence Toland of Los Angeles for treatment of tuberculous peritonitis. There was a moderately advanced, arrested pulmonary tuberculosis. During the three months previous he had had an irregular temperature up to 101°, and considerable abdominal distress with gradually increased swelling of the abdomen. At the time he entered our sanatorium his temperature was 101° to 103°, and the abdominal pain and distress were so great that he was very uncomfortable and unable to sleep without sedatives. He had no appetite, and had been losing a pound a day for the previous two weeks. On May 28, 1924, we aspirated 1800 cc. of a deep-amber-colored serous fluid and injected 400 cc. of oxygen. The patient immediately felt much more comfortable, was hungry for his dinner that evening, and slept through the night without sedatives. Six days later we again aspirated 1800 cc. of a slightly cloudy, brownish, serous fluid, and injected 800 cc. of oxygen. Following this injection the patient's symptoms disappeared, and he has been free from symptoms since. There was a marked general improvement, and he gained in weight at the rate of three to four pounds a week. We were still able to make out the presence of masses in the abdomen. On June 17th, 800 cc. of oxygen were injected, no fluid being present. Again on July 28th, 600 cc. of oxygen were injected. At the end of fourteen weeks from the time treatment was begun

the abdomen was normal, and the patient appeared perfectly well in every way, and had gained seven pounds above his normal weight. At this time he was allowed to return to his home in Los Angeles, where he was advised to follow out more or less the regimen he had learned, gradually increasing his exercise. On December 1, 1924, seven months after beginning treatment, this patient returned to work at a filling station where he has continued and has remained perfectly well.

The injections were as follows:

May 28, 1924, 400 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
June 3, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 6 to positive 7 cm. water pressure.
June 17, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 5 to positive 7 cm. water pressure.
July 28, 1924, 600 cc. oxygen, beginning reading 0, end reading positive 3 to positive 7 cm. water pressure.

Case 3: A young married woman, aged 25, also referred by Dr. Toland for the treatment of tuberculous peritonitis. She came to our clinic July 17, 1924. There was an arrested lesion in both apices. The abdomen was markedly distended by hard masses. The patient had a history of having been acutely ill for about six weeks. During that time she had complained of "biliousness," severe pain in the epigastrium, loss of appetite and inability to take food. On July 18th, with the patient sitting upright, I went through the lower abdominal wall at three different places, but was unable to find any free space. Finally I punctured the abdomen for the fourth time an inch above and an inch and a half to the right of the umbilicus. There was no evidence of fluid, but I was able to inject 300 cc. of oxygen at this point. I saw the patient that evening at 7:30. She was resting comfortably in bed. She stated that she had eaten her supper with a relish, notwithstanding the fact that it consisted of a general tray. She said, "Whatever was done to me this afternoon has taken away all my discomfort, and I feel perfectly well and ready to go home." Five hundred cubic centimetres of oxygen were again injected on July 24th, August 7th and August 15th, respectively. The distention of the abdomen became markedly less, and the masses much less apparent. At the end of four weeks, owing to financial difficulties, the patient returned to her home. She continued on the rest-cure there. Four weeks later she returned to our office. There was still some evidence of masses in her abdomen, and she was advised to return the following week for another injection. At that time her husband was seriously hurt in an automobile accident, and she went to work as housekeeper and nurse, and has continued at work since and at the present time is apparently well.

The injections were as follows:

July 18, 1924, 300 cc. oxygen, beginning reading 0, end reading positive 6 to positive 8 cm. water pressure.
July 24, 1924, 500 cc. oxygen, beginning reading 0, end reading positive 9 to positive 10 cm. water pressure.
August 7, 1924, 500 cc. oxygen, beginning reading 0, end reading positive 11 to positive 12 cm. water pressure.
August 15, 1924, 500 cc. oxygen, beginning reading 0, end reading positive 9 cm. water pressure.

We have also treated 7 other cases of tuberculous peritonitis. One was a patient with far-advanced active pulmonary tuberculosis with marked ascites, who was *in extremis* when first seen. Three thousand cubic centimetres of ascitic fluid were aspirated and 1200 cc. of oxygen injected. The patient felt better for the forty-eight hours following the operation, but at the end of that time went into collapse and shortly afterward died. Another of the 7 cases had been operated on for tuberculous peritonitis, but still suffered rather marked symptoms. She was given one treatment in the Los Angeles General Hospital. The following week she had left the hospital and could not be located, but the interne stated that she had shown marked relief from symptoms. The other 5 cases are still under observation and apparently had completely recovered, the recovery being almost as rapid as in the 3 cases reported in detail, with the exception of 1 case, which was more of the chronic type and which necessitated treatment over a prolonged period.

The treatment of this case was as follows:

November 2, 1923, 350 cc. oxygen, beginning reading 0, end reading positive 4.5 cm. water pressure.
November 12, 1923, 350 cc. oxygen, beginning reading 0, end reading positive 3 cm. water pressure.
November 19, 1923, 450 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
November 26, 1923, 500 cc. oxygen, beginning reading negative 0.5, end reading positive 2 to positive 4 cm. water pressure.
December 7, 1923, 500 cc. oxygen, beginning reading 0, end reading positive 1.5 to positive 2.5 cm. water pressure.
December 17, 1923, 600 cc. oxygen, beginning reading 0, end reading positive 2 to positive 2.5 cm. water pressure.
January 2, 1924, 850 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
January 11, 1924, 1050 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
January 17, 1924, 1100 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.

January 24, 1924, 1200 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
January 31, 1924, 900 cc. oxygen, beginning reading negative 0.5, end reading positive 3 to positive 4 cm. water pressure.
February 7, 1924, 1100 cc. air, beginning reading 0, end reading positive 4 to positive 5 cm. water pressure.
February 15, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 3.5 to positive 4.5 cm. water pressure.
February 21, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 2.5 to positive 3.5 cm. water pressure.
February 29, 1924, 1200 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4.5 cm. water pressure.
March 6, 1924, 600 cc. oxygen, beginning reading 0, end reading positive 1 to positive 1.5 cm. water pressure.
March 14, 1924, 1200 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
March 25, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
April 2, 1924, 1200 cc. oxygen, beginning reading 0, end reading positive 3 to positive 4 cm. water pressure.
July 28, 1924, 800 cc. oxygen, beginning reading 0, end reading positive 2 to positive 2.5 cm. water pressure.
September 24, 1924, 700 cc. oxygen, beginning reading 0, end reading positive 2 to positive 3 cm. water pressure.
December 15, 1924, 500 cc. oxygen, beginning reading 0, end reading positive 2 to positive 2.5 cm. water pressure.

One of these 5 cases was on the service of Dr. E. Richmond Ware at the Los Angeles General Hospital. It was an ascitic form of tuberculous peritonitis. We performed the first aspiration and inflation. Following that the case was treated by Dr. Ware. Of these 7 cases 2 were classed as of the exudative type, 2 of the nonexudative type with either an absence or only slight evidence of masses in the abdomen, and 3 of the nonexudative type with large masses in the abdomen.

We have treated 6 cases of intestinal tuberculosis by oxygen inflation of the abdomen. Four of these were of the ulcerative type and have been previously reported by my associate, Dr. Gleeten (14), while 2 were of the stenotic-hypertrophic type. These cases were all cases of far-advanced active pulmonary tuberculosis, and had been treated by diet and medication, including calcium chloride intravenously, without relief. In the ulcerative cases the diarrhea was reduced from ten to twenty movements a day to two or three; the appetite improved and there was a general feeling of improvement and ability to rest and sleep. In the stenotic-hypertrophic type, outside of a general stimulation and an improvement which could be credited to the psychic influence, there was no change. Of the 2 cases of abscess treated in our clinic by this method one was a deep gluteal abscess secondary to a small focus in the spine. An aspirat-

ing needle was inserted to a depth of 3 inches before the abscess was encountered. Eight hundred cubic centimetres of tuberculous pus were aspirated. The abscess cavity was then washed out with iodine solution, consisting of 1 dram tincture of iodine to a pint of sterile water, and oxygen injected to the rather marked distention of the cavity. The needle was withdrawn and a purse-string suture tightly tied around the needle track. This case was treated a year ago, receiving only one treatment. There was almost an immediate disappearance of symptoms, and there has been no recurrence of the abscess. The second case was a large fluctuating tuberculous abscess of the neck. Two needles were inserted, one above and one below. Four ounces of tuberculous pus were aspirated through the lower needle, and the iodine solution run in through the upper needle and allowed to flow out through the lower needle until the cavity was thoroughly washed out. The lower needle was then withdrawn, and a purse-string suture tightly tied. Oxygen was injected until the cavity was mildly distended, and the upper needle withdrawn, and a purse-string suture drawn tight. A week later the cavity was again filled with pus. The operation was repeated. Following this the pus failed to reappear and the abscess cavity closed without any further trouble.

In the treatment of these abdominal conditions, especially that of peritonitis, when the abdomen is not opened there is always a question as to the diagnosis. This was particularly emphasized in one of our cases not included in this report. In the Los Angeles General Hospital a girl fourteen years old, with the diagnosis of far-advanced pulmonary tuberculosis, Pott's disease and tuberculous peritonitis with ascites, was transferred to my service for the treatment of peritonitis. On general observation she was markedly cachectic, and was not what you would consider a typical picture of tuberculosis. Thirty-eight hundred cubic centimetres of clear serous fluid was aspirated and 800 cc. of oxygen injected. The fluid rapidly returned to the original amount. The operation was repeated five times with the same result each time. The patient grew steadily worse and died. Autopsy showed a generalized carcinomatosis of unknown origin, with no evidence of tuberculosis in any of the organs.

The cases included in this report were all seen by a number of different clinicians and had been kept under observation for a long period, so that we felt that the diagnosis in all the cases was reasonably certain.

In the preliminary report of this work, the contraindications, risks

and the possible method of action of the oxygen were discussed in detail. Time does not permit me to review them now.

While I do not consider oxygen inflation a cure-all for all of these different conditions, in which tuberculosis exists in closed cavities, I do believe that, in the types of tuberculous peritonitis which may be considered amenable to treatment, in general this method is perhaps the simplest and most efficient that we can employ, and will greatly shorten the period ordinarily required for treatment. In the more severe types, in which the peritonitis is the dominating disease, whether it be of the exudative or nonexudative type, provided the general condition of the patient is fairly good, or when the lung condition is slight or more or less inactive, this method of treatment will very often effect a cure when other methods would not. Even in the more advanced or complicated cases of peritonitis I believe that the simplicity of this method, as well as the palliative effect, warrants its being tried out.

In the ulcerative type of tuberculous enteritis, in a great number of advanced cases, it is the most efficient means we have of relieving symptoms, and if used in the early cases it may be instrumental in effecting a cure.

In tuberculous abscesses, in general, of the more acute forms, which are not complicated by sinuses or mixed infections, it is, I believe, a very efficient and effective method of treatment.

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PULMONARY HILUM TUBERCULOSIS IN THE ADULT[1]

HAROLD FREED AND J. B. WEBB[2]

Medical literature is replete with detailed and careful studies of various phases of tuberculosis of the tracheobronchial lymph nodes in the child. This subject has especially claimed the attention of numerous investigators in the fields of both periatrics and phthisiology. However, the same condition in the adult has been very inadequately studied. This fact is our justification for reporting the results of our investigation.

The material that forms the basis of our study is drawn from a group of patients observed during a period of from two to five years, a group affected with the rather unusual form of intrathoracic tuberculosis mentioned. Our sources are the files of the claimants of the United States Veterans Bureau, District 14. These contain complete records of frequent periodic physical examinations of the patients with detailed laboratory findings, all extending over a period of several years. All the cases reported had periods of hospitalization in government institutions for the correct diagnosis of their conditions. In addition to being hospitalized, these cases were examined semiannually at the field offices of the Bureau. Thus it may be noted, incidentally, that in the report of these cases is contained a feature which the National Tuberculosis Association has been long striving to foster; that is, a thorough and systematic follow-up of each individual patient.

The term *adult tracheobronchial gland tuberculosis* may be defined as a tuberculous adenitis of the tracheobronchial lymph nodes located at and around the bifurcation of the trachea, characterized by caseation and subsequent discharge of the tuberculous material to the outside, without any demonstrable involvement of the parenchyma of the lung, and with the same clinical manifestations as those found in pulmonary phthisis. The first mention of tracheobronchial gland tuberculosis of the adult is that of J. N. Hall (1), who in 1903 reported three

[1] Published by permission of the Medical Director, U. S. Veterans Bureau.

[2] U. S. Veterans Bureau, San Antonio, Texas.

cases in which the symptomatology and physical findings were those of our patients. He did not, however, have at his disposal the roentgenological confirmation of his findings, which was of such great aid to us in excluding any parenchymatous pulmonary involvement and in demonstrating any possible hilum adenopathy. The symptom complex of his cases included loss of weight, persistent daily elevation of temperature, purulent expectoration at times positive for tubercle bacilli and small pulmonary hemorrhages.

The findings in our series of 9 cases paralleled those of Hall's and the diagnosis was the same, with the exception that it was considerably reinforced by successive radiograms of the chest.

REPORT OF CASES

Case 1: B. H., white, male, 30 years old; accountant by profession. In the service for three years, from 1916 to 1919. *Family history* negative. *Past history:* measles, typhoid fever, smallpox in childhood; pneumonia 10 years ago. *Chief complaints:* pains and aching in the chest, occasional elevation of temperature, loss of weight, dyspnea upon moderate exercise. Patient complains of these symptoms ever since discharge from the service.

First examination by the Bureau in November, 1923, shows general negative findings, except for a spherical shadow about 5 cm. in diameter at the hilum, extending to the right of the heart, seen on the X-ray plate. The hospitalization record of April, 1924, of the William Beaumont General Hospital states that the patient was ambulant, but running a slight fever with a slightly accelerated pulse. Repeated examination of the chest failed to reveal any râles or other signs sufficiently definite for localization of any tuberculous changes. Certified sputum examinations, three out of eight, revealed the presence of tubercle bacilli. A radiogram of the chest showed no changes in the parenchyma of the lung, but an oval shadow in the right hilum which, in the opinion of the attending medical officer, was in all probability "a tuberculous focus which discharges intermittently into a bronchus." Subsequent examination by the Bureau on November 10, 1924, showed the man markedly underweight, with a persistent elevation of temperature reaching 37.5°C. and a pulse of 100. The sputum examinations as well as the physical examination of the chest at the time were negative.

Case 2: H. J., white, male, 28 years old; nurse by profession. Served in the Medical Corps of the United States Navy from 1917 to 1919; while in service had German measles and two attacks of uncomplicated influenza. Family and past history negative. The onset of respiratory trouble dates from 1919, when he began to notice some cough, loss of weight, night-sweats, and pains in the

chest. His physician at that time pronounced him a case of active pulmonary tuberculosis upon finding tubercle bacilli in his sputum. He went West for a year and improved slightly.

The first examination by the Bureau on May 24, 1923, showed a poorly nourished, anemic man, who complained of shortness of breath upon slight exertion, pains in chest, cough, evening fever, general weakness, and loss of weight. Physical examination report states height 6 feet ¾ inch, weight 159 pounds, temperature 99.1°, pulse 84; general physical examination negative. Physical examination of chest is essentially negative; no marked changes from normal on inspection, palpation and percussion; auscultation normal; no râles. Chest radiogram reveals no traces of tuberculous involvement. Sputum is negative for tubercle bacilli. Examination in April, 1924, reveals same findings, patient still running a low-grade fever in the afternoon, ranging from 99.2° to 99.4° F., with a pulse of about 100. He lost 3 pounds more and still has the same complaints, except that he has periods of increased cough, expectoration, and elevation of temperature above his usual normal. Chest radiogram taken in April, 1924, is identical in appearance with that taken the year previous and shows no visible pathological changes. Several certified specimens of sputum show a great number of tubercle bacilli.

Case 3: C. N. F., white, male, 33 years old; clerk by trade. Was in the service from 1916 to 1918. Record of the first examination by the Bureau in 1921 shows a poorly nourished man, complaining of pain in the cardiac region, dyspnea, expectoration of mucopurulent sputum, cough and loss of weight. Examination of the chest was essentially negative, with the exception of a few musical râles scattered throughout the chest. No tubercle bacilli were found in the sputum. Subsequent examination by the Bureau on July 26, 1923, shows the man with about the same weight as in 1921; temperature 37.1°C, pulse 114. Physical signs in the chest were negative. Temperature taken on four consecutive days was 99.8° in the afternoon, pulse 104 to 112. Certified specimens of sputum show tubercle bacilli, many to the oil-immersion field. X-ray of the chest taken four months later shows a picture not typical of pulmonary tuberculosis but hila rather large and cottony, and containing numerous flocculent areas involving the lymph nodes.

Case 4: J. F. C., white, male, 29 years old, mechanic by trade. Was in the service from 1918 to 1920. Family and past history up to date of enlistment negative. Had pneumonia while in service; was discharged on account of inactive pulmonary tuberculosis and chronic articular rheumatism, probably gonorrheal in origin.

The file of this man contains records of numerous physical and laboratory examinations, with repeated chest radiograms extending over a period of

three years prior to the present date. His complaints through all this period were general weakness, tired feeling, slight expectoration, cough, persistent elevation of temperature and loss of weight. First examination of this man (February, 1920) shows signs of entire absence of pathological changes in the chest. Chest radiograms revealed peribronchial thickening; otherwise negative. Temperature and pulse normal, sputum negative. Repeated physical examinations for the past four years in the United States Veterans Bureau hospitals and suboffices uniformly present the same findings. The man is anemic, emaciated, 5 feet, 11 inches in height; weight varies from 110 to 125 pounds; temperature fluctuates between 98.6° to 101° F., with pulse about 100 at rest. Physical signs in chest essentially negative. Râles were found at no time. Radiograms of the chest are negative as to parenchymatous lung lesions. Sputum negative for tubercle bacilli on smear examinations until 1924, when certified specimens of sputum showing tubercle bacilli were obtained on two different occasions in the United States Veterans Hospital no. 93, Legion, Texas.

Case 5: G. G. S., white, male, 30 years old, railroad switchman. Was in the service six months in 1918. Family and past history up to enlistment negative. Had attack of uncomplicated influenza while in the service and has never felt well since.

Upon discharge he returned to his old occupation of railroad switchman, but was unable to keep up with his work as in the past. In November, 1921, he collapsed while at work and was carried home. His physician then made a diagnosis of pulmonary tuberculosis. One month prior to his collapse he married. Wife has pulmonary tuberculosis now. First examination in file dates from July, 1923. The record shows a poorly nourished, anemic man, complaining of pain over his heart, dyspnea on exertion, cough, night-sweats, and loss of weight. Physical examination shows height 5 feet, 9 inches, weight 126 pounds, temperature 99.3°F., and pulse of 168 at rest; general physical examination negative. Neuropsychiatric examination and basal metabolism test did not reveal any neurological condition. Urinalysis, feces, blood count, and blood Wassermann were all negative. Certified specimen of sputum showed tubercle bacilli. Physical examination of the chest revealed no deviation from the normal. Chest radiogram report stated "No evidence of existing tuberculosis." Several examinations since that date do not reveal any change in the physical, clinical, or laboratory findings. Tubercle bacilli in the sputum have been present on numerous occasions.

Case 6: L. T. S., white, male, 28 years old, mechanic by trade. Was in the service from 1917 to 1919. While in the service he was treated for pulmonary tuberculosis, chronic, inactive. He began to notice in May, 1920, loss of

weight, weakness, night-sweats, severe cough, and expectoration of blood-streaked sputum. Examination record of the United States Veterans Hospital, no. 55, showed that the man is slightly underweight; temperature and pulse not recorded. There were no physical signs in the chest indicating tuberculosis. X-ray examination of the chest also revealed no evidence of pulmonary lesions. Subsequent examinationss on file confirm these findings, notably that of United States Veterans Bureau Hospital, no. 51, of June, 1922. The physical and X-ray examinations of the chest are still negative, but the patient is markedly under weight (height 70½ inches, weight 118 pounds) and is running a temperature of 37.2°C. and pulse of 103. Examination of October 20, 1923, shows the patient running a temperature above 99°F., with a pulse above 100. The physical signs in the chest and X-ray findings are negative. At that time, however, the sputum was positive for tubercle bacilli on four different occasions, all the specimens being certified. The patient is still very toxic, losing steadily in weight, and has had three small pulmonary hemorrhages recently. He complains of excessive expectorations, purulent, blood-streaked, and at times fetid. The last examination in file, that of November, 1924, reports the same negative chest findings, sputum still negative for tubercle bacilli, and X-ray diagnosis from William Beaumont General Hospital reading, "No definite radiographic evidence of parenchymatous involvement, either lung, noted."

Case 7: L. H., colored, male, 30 years old, porter. Was in the service three months in 1918. He was discharged on account of tuberculosis, pulmonary, chronic, arrested. Family history negative. *Past history:* Malaria, mumps in childhood, winter coughs for past three years, dyspnea on exertion.

About a dozen physical examinations of the chest with laboratory data and chest radiograms, extending from November, 1920, to date, are on file. Subjective complaints show little variation during the last four years; troublesome cough, worse at night; occasional hemoptysis, loss of weight, and pains in the chest. The physical examination of the chest reveals no pulmonary involvement except that on one of the examinations in 1921 a friction rub at the base of the right lung was heard. Another examination, that of May 10, 1923, states that a few, fine râles were heard at the apex of the left lung. Urinalysis, sputum, feces, blood count, and blood Wassermann are all negative. Physical examinations of November 6, 1923, December 27, 1923, and October 3, 1924, show that restricted mobility and slight increase in resonance and fremitus are present in both upper lobes; but no râles or other abnormalities were noted. X-ray reports on the numerous chest radiograms taken since November 17, 1920, mention very markedly accentuated and thickened hilum shadows. The X-ray report of November 6, 1923, states that the hilum is so markedly accentuated as to suggest a process such as syphilis or Hodgkin's disease. The patient is quite underweight; some examinations find him with a low-grade

temperature and a slight acceleration of pulse, although most of the pulse and temperature records were normal. Sputum has been negative ever since his discharge from the service until October, 1924, when one certified and one uncertified sputum examination each revealed the presence of tubercle bacilli. Lately he has had an occasional slight rise of temperature, with one slight hemorrhage from the lungs.

Case 8: J. W. R., white, male, 33 years old, tailor. In the service for eight months during 1918. Discharged because of malnutrition and weakness, with the diagnosis of probable pulmonary tuberculosis. The man was first examined by the Bureau in December 1919, at which time he complained of weakness and slight cough; physical examination gave practically negative results, except that the man was of sallow complexion and undernourished. A diagnosis of mild chronic bronchitis was given. One and a half years later he presented himself for examination, complaining of weakness, inability to do any work, pleuritic pains in both sides of his chest, and dyspnea. The report of the examiner shows that the man is about 25 pounds underweight. Physical signs in the chest essentially negative. Sputum, blood Wassermann, urine, feces and complete blood count negative. Temperature range 97.6°F.–101.8°F., with pulse of 70–100. Chest radiogram negative. Three months later in October, 1921, the patient had two slight pulmonary hemorrhages, but not until September, 1924, were any tubercle bacilli found in his sputum, when smears from a certified specimen showed the slide heavily seeded with tubercle bacilli. The examination report a month later in June, 1925, presented the same picture,—numerous tubercle bacilli in the sputum with the physical signs and X-ray findings of the man's chest negative for any pathological changes.

Case 9: H. B. B., white, male, 30 years old, boiler-maker. Was in the service for one and a half years during the World War; had influenza complicated by simple pleurisy while in the service; chancroidal (?) infection in 1920, for which he received local treatment and a course of salvarsan injections. The first examination by the Bureau in November, 1920, showed the man markedly underweight, 132 pounds instead of his normal weight, 180 pounds, and complaining of slight cough, expectoration and weakness. Physical examination revealed no abnormalities in the man's chest. The chest radiogram was also negative. Blood Wassermann, ++; urinalysis, complete blood count, temperature and pulse record within normal limits. The patient's condition prevented him from doing any work and he felt himself continually declining. In July, 1924, he was admitted to United States Veterans Bureau Hospital no. 50 for diagnosis and treatment. At that time he complained of a slight cough and pains in the chest, night-sweats, and expectoration of purulent matter which at times was more profuse than at others. Physical signs of

pathological changes in the man's chest were entirely lacking, and chest radiogram failed to reveal any parenchymatous or hilum involvement. Urinalysis, feces, and blood Wassermann negative, blood count showing only a secondary anemia. Diagnosis of active tuberculosis made on the presence of tubercle bacilli in the man's sputum, demonstrated twice during his stay at the hospital, and on persistent low-grade fever. Examination of the man by us at this office gave the same findings as those of the hospital, but no tubercle bacilli were found in the man's sputum.

DISCUSSION

A review of these cases is interesting from the standpoint of etiology, and at the same time illustrates the difficulty one encounters in obtaining sufficient evidence upon which to base a diagnosis of tuberculous activity.

Genetically, this is probably the most interesting of all the forms of chest tuberculosis. Gekler (2) mentions this condition briefly, and advances three possible explanations of its etiology: (1) it is a recurrence of a primary tuberculous infection of childhood; (2) it is a primary tuberculosis of the adult, who has never before come in contact with the tubercle bacillus; and (3) the primary tuberculosis of childhood resulted "in complete healing, with lapse of sufficient time for any immunity or hypersensitiveness that may have been established to disappear, to be followed later by a reinfection which would behave similarly to the initial infection in early childhood." Gekler believes that the first two processes do actually take place, while the third explanation is rather questionable. From the study of our series of cases it would seem that the primary mode of infection in the adult is the least plausible, as all of our cases were city dwellers, living in an environment which would hardly permit them to escape primary infection in childhood. As most of them had seen quite strenuous service during the war, the lighting up of a latent focus, which had its incipiency in childhood, appears to be the more logical theory. We personally believe that probably no one factor alone will account for the development of this condition; in fact, we accept Krause's dictum (3) that any case of tuberculosis is the summation of a multitude of forces. A determination of the exact nature of these forces, as well as the explanation for the fact that the tracheobronchial lymph nodes in our series showed enlargement and necrosis, while in far-advanced pulmonary phthisis the same nodes are practically uninvolved, is beyond the scope of this article.

The difficulty of diagnosing activity is the other problem presented by these cases. In the work of the medical service of the United States Veterans Bureau the differentiation between an active and inactive tuberculous process takes on a special significance. By Act of Congress the presence of activity is the determining factor in establishing the service connection and compensation status of the Bureau's beneficiary. No effort is being spared in defining the stage of the disease in the different individuals in accordance with specific regulations laid down by the Bureau. In as large a medical organization as that of the Bureau a certain uniformity must be attained in judging the extent of the tuberculous process. The general rule applying is that two or more of the following cardinal findings must be present before the disease be considered active:

(a) Sputum positive for tubercle bacilli.

(b) Pleurisy with effusion.

(c) Cavity or pneumothorax diagnosed by stereogram and physical examination.

(d) Active tuberculous lesion evidenced by definite physical findings indicating a tuberculous involvement, most characteristic of which are typical indeterminate, localized, persistent moist râles (commonly called crepitant and subcrepitant) in the upper lobes, increased or manifested on the first inspiration after a forced expiration followed by a cough.

(e) X-ray findings, of which roentgenograph stereograms manifesting cottony densities, cirrus clouding, or areas of rarefaction surrounded by annular shadows interpreted as cavities, diagnostic of tuberculous infiltration, caseation, or cavitation, are the most important. (Unsupported X-ray findings will not; however, be accepted as conclusive evidence of activity.)

(f) Active tuberculous lesion, evidenced by definite toxemia of probable tuberculous origin, manifested by one or more of the following symptoms: fever, loss of weight, and rapid "resting" pulse.

The tuberculin test in its various forms, the Wildbolz auto-urine reaction, and several of the newer diagnostic methods, such as the red-cell sedimentation rate, are not officially recognized by the Bureau.

The wisdom and scientific value of these regulations may be questioned, but nevertheless they provide us with the best working plan at present. True it is that the great majority of these cases will have two or more of these findings, but this does not always hold good for the tracheobronchial type of tuberculosis in the adult, as appears from the survey of the cases reported in this article.

Numerous accepted signs of tracheobronchial adenopathy (4), such as constant pain in the mammary region associated with interscapular dulness, Schick's "expiratory stridor," "brassy" cough, dilatation of veins over the anterior aspect of the thorax, dilated capillaries in the region of the seventh cervical vertebra, puffiness of face and eyelids due

to venous obstruction, spinalgia, d'Espine's sign; all of these were either not present or not noticed by the examiners in this series. Incidentally, we might note that five of these cases were examined personally by the authors and showed none of the above-mentioned signs.

The only two definite signs which may be present, and which would be helpful in making a diagnosis of active tuberculosis in these cases, are the toxemia of these patients, resembling in every respect that of pulmonary tuberculosis, and the finding of tubercle bacilli in their expectoration. As toxemia, extending over a period of several years, was present quite constantly in our patients, the diagnosis was really contingent upon the presence of tubercle bacilli in their sputum. From the intermittency of the discharge of tubercle bacilli, which forms one of the markedly characteristic features of these cases, one can readily see how the diagnosis of tuberculous activity may be passed over if only a few sputum examinations are made.

We are now comparing at the laboratory of the regional office the concentration method for the demonstration of tubercle bacilli with the smear method, and it seems from the few cases we have on hand that the concentration method is far superior to the smear method. Since the concentration method is very seldom employed in the medical work of the Bureau, or in state, county, city, or private laboratories, the finding of tubercle bacilli in the sputum of these patients is really a matter of chance with the odds against the patient.

SUMMARY AND CONCLUSIONS

Before summarizing the results of our study we may well digress to touch upon the subject of laboratory diagnosis. The patient's sputum should be repeatedly examined by the antiformin concentration method and several guinea pigs inoculated at weekly intervals. Potassium iodide, although strongly condemned by men like Norris and Landis (5), should, in our opinion, be used for diagnosis in these cases. Since we do know that some disintegration of the tracheobronchial lymph nodes at intervals takes place, as evidenced by the increased purulent expectoration laden with tubercle bacilli, and that no extension of the tuberculous process to the pulmonary parenchyma occurs, we should not hesitate when the establishment of the diagnosis rests with the demonstration of tubercle bacilli in the sputum, and when we are certain that there is no pulmonary involvement, to avail ourselves of the iodides in the usual doses. The breaking down of a superficial tuberculous

focus in these lymph nodes will only be hastened, and the finding of tubercle bacilli will be materially facilitated. An examination of the bile obtained by the Lyon-Meltzer method, as described by one of us (6) for the demonstration of tubercle bacilli, is advisable. The procedure is a comparatively simple one and may be of considerable aid in this type of case.

By way of conclusion we will record our belief that in any case with such toxic symptoms as loss of weight, purulent expectoration, persistent elevation of temperature above 99°F., acceleration of the "resting" pulse to over 90 per minute, the subjective symptoms pointing to chest involvement in the absence of a definitely established cause for the toxemia, tuberculous disease of the tracheobronchial lymph nodes should be considered as a possibility. In the event that we do find tubercle bacilli at one but not at subsequent examinations, the patient should be considered actively tuberculous, if there is a definite toxemia, even in the absence of physical and radiographic findings of chest disease.

Briefly, the salient features of the cases reported are: (1) Marked chronic course of the disease; (2) negative physical and X-ray findings with the exception of the presence of marked enlargement of hilum shadows in 3 out of 9 cases; (3) peculiar localization of the changes in the lymph nodes, as evidenced by the lack of extension to the lung parenchyma during a course of observation extending over several years, when the patients showed distinct signs of tuberculous activity; (4) occasional finding of tubercle bacilli in the sputum; and (5) objective and subjective clinical symptoms of tuberculous toxemia identical with that of pulmonary phthisis.

The authors wish to thank Mr. J. Werne, senior medical student of Baylor University, for assistance rendered in the preparation of this paper.

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REMARKS ON "OCCULT" OR "CONCEALED" TUBERCULOSIS

EVERETT K. GEER[1]

A recent book by Head (1) on *Concealed Tuberculosis* and three articles by Warfield (2) dealing with "Occult Tuberculosis," coming, as they do, from men of no little prominence, should and do evoke discussion.

It is generally admitted that there is a time in the development of pulmonary tuberculosis when the lesion is not demonstrable; we cannot detect it by physical signs nor does the roentgen ray give us positive evidence; and repeated sputum examinations, if there is sputum, are persistently negative. Early cases of hemoptysis and "idiopathic" pleural effusions furnish us highly suggestive evidence that a pulmonary lesion may be active and yet not demonstrable, in the sense that we cannot say there are medium râles in one or both apices or a parenchymatous roentgen ray lesion in one or another interspace. And so there must be cases which, if found early enough, have only the symptoms and signs of chronic intoxication. The book and articles mentioned above are attempts to solve this problem, for such it is, and one of which the phthisiologist is fully cognizant, and for the solution of which he will be profoundly grateful.

The elucidation of this difficult question is placed by Warfield and Head on an extremely simple basis by granting to the tuberculin reaction practically the same specific qualities which the Wassermann reaction is presumed to have in the diagnosis of syphilis. Head makes the rather astounding and highly debatable statement that, "Postmortem and clinical experience has proved the reliability and specificity of a positive subcutaneous tuberculin test as proof of the existence of an active tuberculous infection."

His average diagnostic injection of 4 mgm. (with no really accurate reason for it being that amount) is more than enough to give the majority of thoroughly sound people a day or two of febrile discomfort. Until he can explain why perfectly well people who give a general reaction to tuberculin are not otherwise sick and not in need of treatment, it must be

[1] Pokegama Sanatorium, St. Paul, Minnesota.

considered illogical to assume that people with symptoms only of chronic poisoning and unexplainable "dyspepsia" are actively tuberculous, should they happen to react to subcutaneous tuberculin. The analogy of the value of the tuberculin reaction to the virtue of the Wassermann reaction is far fetched,—too much so to be accepted. The pathology and immunology of tuberculosis and syphilis digress too markedly to permit the validity of this comparison. Syphilographers are rather unanimous in the opinion that the majority of persons with positive Wassermann reactions should have antiluetic treatment. But heaven forbid any such muddled state of reasoning which would induce phthisiologists to advice the standard treatment of to-day for all those with positive tuberculin reactions.

Warfield, on the other hand, is an exponent of the specific attributes of the intracutaneous tuberculin test. And here one must wonder why 0.1 mgm. is the dividing line between diagnoses of active and inactive tuberculosis, and why a reaction 2 cm. in diameter is not just as much an expression of active disease as a 4-cm. reaction would be. Indeed, his reasoning is far from convincing.

Both Warfield and Head are very skeptical about our neurological colleagues, who entertain diagnoses of neurasthenia, psychasthenia, gastric neuroses, etc. The question suggests itself: What would their own diagnosis have been in their reported cases, had not the self-explaining tuberculin reaction manifested itself? That there are such cases and not a few I shall show presently.

A further question arises: Have not these men, intentionally or unintentionally, avoided the fact that many people are to-day not equal to the stress and strain of our modern commercial, industrial, and pleasure-loving existence? Are we asked to believe that nervous instability and exhaustion are myths? Certainly the affirmative answer to these interrogations is strongly suggested by the contention that a positive tuberculin reaction clears away the mists of etiological doubt and reveals many of these disabilities firmly ensconced upon the pedestal of tuberculous disease.

The argument appears to be a recrudescence of bygone days. Just as the therapeutic value of tuberculin is again being discussed so is its diagnostic worth. Clear thinking is indeed needed, or else our sanatoria will be overflowing with neuropsychiatric patients who are unfortunate enough to have reacted intracutaneously or subcutaneously to tuberculin.

What, after all, is the true significance of the tuberculin skin test? It means that the tissues are reacting in a different manner to the products of the tubercle bacillus by reason of previous infection with the bacillus. What does intensity of that reaction signify? In the presence of demonstrable anatomical tubercle does it necessarily mean an active tubercle? Knowing the course of the allergic reaction, as shown by Krause, as one which may begin within a week after infection, rise in intensity to its peak and then gradually subside almost to the base-line, one is tempted to interpret a sharp skin reaction to tuberculin in the absence of manifest tuberculosis as bespeaking *recent* infection rather than *active* disease, whether this recent infection be from without or represent the extension of an already present focus. And, most certainly, recent infection has occurred to large numbers of us and is still taking place without manifestations of a diseased condition. Witness the case of L. A. H., who had been sleeping with his brother for six months up to the time the brother was found to be actively tuberculous with positive sputum. The intimate contact warranted an examination, but I could find nothing abnormal, objectively nor subjectively. He was clinically sound. Yet to 0.1 mgm. O.T., intracutaneously, he had a 4.5-cm. reaction, with local swelling and heat. Active tuberculosis? Most certainly not. But an exhibition *par excellence* of *recent infection* it assuredly is.

It must be granted that a general reaction, following a small amount of tuberculin subcutaneously, probably means that there are some tubercles in the body, which are not any too well invested with a fibrous capsule. The fact that a focal reaction must precede a general reaction leads to that conclusion. And, further, this general reaction undoubtedly warrants close observation over a period of several months. But the advocates of the specificity of the tuberculin reaction, as applied to the activity of a lesion, must explain why one patient's tubercles, which give a sharp enough focal reaction to a given amount of tuberculin to produce a general reaction, do not cause ill health while another's, under the same conditions, supposedly do. One hears, "Difference in virulence of infecting organisms," "Difference in reaction of host," etc. But these are insufficient and poorly explained generalities.

I now come to the obvious, time-worn, yet timely contention that the great value of tuberculin used diagnostically in adults is from the negative side. It is universally admitted that clinically tuberculous persons, provided they are not moribund or suffering from intercurrent affections,

such as influenza, measles, etc., are extremely sensitive to very small quantities of tuberculin. If, then, a suspected case with negative findings can be given comparatively large amounts (up to 10 mgm.) of tuberculin with no general reaction, it is reasonably safe and highly rational to conclude that whatever tubercles they harbor must be competently invested by fibrosis and therefore incapable of producing symptoms. Well-fibrosed tubercles, which are incapable of giving systemic reactions to irritation from tuberculin, are surely *not* doing so in the absence of that annoyance. Lawrason Brown and his associates have used tuberculin diagnostically, based on these contentions, with success, as have many others, and my limited experience has been confirmatory of their larger one.

It may be erroneous, though it is perhaps probable, to assume that men doing tuberculosis work do not see these elusive cases which confuse our diagnostic efforts. Take such cases as the following:

D. O., female, seen October 6, 1924, single, age 17, high school student. *F. H.:* Father died of pneumonia. Mother living and well. No tuberculosis in family. *P. H.:* Measles in childhood. Several attacks tonsillitis to 1919, when her tonsils were removed. Influenza 1921. Appendicectomy. *P. I.:* Was well up to December, 1923. Appetite became poor and she lost weight. Became nervous and irritable. Was in hospital three weeks in June, 1924, with "nervous breakdown." Then went to country for summer when she had chest discomfort which was called "pleurisy." In September, 1924, was told she had pulmonary tuberculosis, and was sent to a sanatorium where she stayed only a week. Returning to St. Paul she came under my observation in October, 1924. Complained chiefly of loss of strength and nervousness. No cough or expectoration. Never spat blood. No digestive disturbance except constipation. Weight 90 pounds, average not known but had weighed as high as 115 pounds in January, 1924. Temperature normal always and pulse varied from 60 to 76. General physical examination revealed only a rather tall, slender, fair girl, of asthenic type, with no abnormal physical findings. Chest long, narrow and flat, and showed only a possible slight narrowing of the left apical field. Stereoscopic films of chest quite clear. Urine normal; blood examination revealed hemoglobin 84 per cent; *r. b. c.*, 3,860,000; *w. b. c.*, 4,450 with normal differential. Wassermann negative.

Was given 1-3-5-10-10 mgm. O. T., subcutaneously, every four days with no reaction except very faint local one.

She was told that she had no tuberculosis.

A. M., male, married, age 49, engaged in railway mail service, entailing long, irregular hours. Seen March 28, 1925. *F. H.:* Unimportant. Wife and 3 children living and well. No tuberculosis. *P. H.:* Usual childhood diseases. Always well up to three years ago. Had grippe in 1923. *P. I.:* Felt well to March, 1922. Began to be run-down and tired easily. Lost weight gradually. Took colds easily and has coughed only at those times. At present, complains of cough with scanty mucoid expectoration; poor strength, nervousness, inability to sleep well, epigastric pain, 15 pounds loss in weight. Had been told he had pulmonary tuberculosis and was referred for sanatorium. Examination revealed tall, thin man of asthenic type, greatly worried over his condition. Only abnormal physical findings noted were

that muscles over left apex seemed smaller than those over right, and a moderately enlarged prostate. Stereoscopic films of chest showed increase in linear markings, but no definite parenchymatous infiltration. Blood-pressure 150/80. Concentrated 24-hour sputum specimens always negative.

Urine normal. Wassermann negative. Hemoglobin 68 per cent; *r. b. c.*, 3,840.000; *w. b. c.* 8,856. Ewald meal showed 50 cc. creamy, fairly well-digested material, with free HCl 46 and total acidity 66. Stools showed no occult blood. Roentgen-ray examination of gastrointestinal tract showed no abnormality. He was given 1-3-5-10-10 mgm. O. T. at four-day intervals with no general reaction and a very moderate local one.

He was told that he had no tuberculosis, but that his gastric complaint warranted careful and prolonged observation.

The preceding cases are, as nearly as possible, duplicates of those related by Warfield and Head, with the exception that they did *not* respond to tuberculin. The tuberculin was potent, as it was tried on known tuberculosis patients intracutaneously with marked reactions. It was perhaps luck that all tubercles in these patients were well fibrosed, for their failure to respond certainly furnished adequate grounds, along with the other negative data, to warrant an opinion of a nontuberculous condition. A general reaction in either case would perhaps have confused things slightly, but assuredly not enough to have justified a diagnosis of active tuberculosis, focus concealed.

CONCLUSIONS

1. No form of a tuberculin test yet devised is able to distinguish between tuberculous disease and tuberculous infection.

2. From a diagnostic standpoint, the absence of a general reaction to a comparatively large dose of tuberculin is infinitely more helpful than its presence.

3. To base a diagnosis of occult or concealed tuberculosis upon the positive response to tuberculin, no matter how administered, is unwarranted, not only because of what we know concerning the epidemiology of tuberculosis but because of the fundamental facts underlying the tuberculin reaction.

4. The solution of the occult or concealed tuberculosis problem is not to be found in any one test, but will be revealed only by searching and repeated examinations, and prolonged observation.

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CHRONIC MILIARY TUBERCULOSIS OF LUNGS WITH RESOLUTION

A Case Report

I. S. KAHN

Miliary tuberculosis is not invariably rapidly fatal. Somewhat similar cases to the present one have been reported (1), (2), (3). However, the rarity of even partial resolution of this form of tuberculosis warrants the report of the following case.

C. H. H., age 44, single, real estate operator, was first seen February 2, 1924. He gave a history of cough, expectoration, fever and loss of weight for several months, with increasing shortness of breath on exertion, and recent abdominal distention without pain. Past history unimportant except for pericarditis one year previously with probable tuberculosis at that time.

Physical examination revealed an emaciated, dyspnoeic, rather sick individual. The temperature was 101°, pulse 108, respirations 28. His height was 72 inches, weight 101½ pounds.

The abdomen contained considerable fluid and measured 32 inches in circumference at the umbilicus. There were no areas of tenderness and no palpable tumor masses. Slight oedema of the shins and ankles was present.

The right lung showed impaired percussion resonance throughout, with universal indeterminate râle formation. The left lung showed similar universal râle formation, with marked depression of the entire anterior thoracic wall. This appearance simulated what is seen in an old chronic empyema. Decided percussion flatness and diminished breath-sounds existed over the precordia, entire axilla and from the base posterior to a level with the lower scapular angle. There were no adventitious cardiac sounds.

The sputum was positive for tubercle bacilli. The blood was negative except for a moderate secondary anemia. The Wassermann test was negative. The urine and feces were negative.

X-ray pictures of the chest, February 2 and April 22, 1924, showed diffuse universal bilateral confluent miliary tuberculosis with pericarditis.

No attempt was made to determine the character of the abdominal exudate.

A practically hopeless prognosis was given. However, an extreme hospital rest-cure regimen was instituted, with sunlight exposures to the chest and abdomen. Sodium cacodylate, 3 grs., was given hypodermatically twice a week. Much to my surprise, within a few weeks the peritoneal fluid dis-

appeared completely without tapping, and the temperature commenced to fall, with rapid diminution of cough and expectoration, disappearing completely in four months' time. By that time the chest râles were greatly reduced, with coincident marked improvement in the patient's general condition. After an eight months' hospital residence, as a strict bed case, this patient left for his home in another southern state. The X-ray of the chest at that time showed some clearing of his process.

Rest cure was continued at home reasonably efficiently.

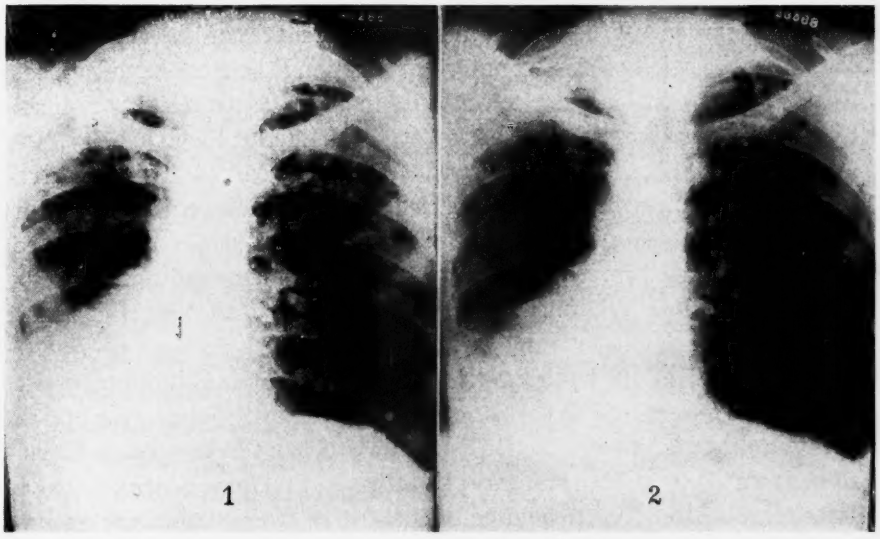

FIG. 1. APRIL 22, 1924. TYPICAL HEAVY CONFLUENT GENERALIZED MILIARY TUBERCULOSIS OF BOTH LUNGS. PERICARDITIS

FIG. 2. APRIL 29, 1925. MARKED CLEARING OF TUBERCULOUS PROCESS IN RIGHT LUNG. LEFT LUNG PRACTICALLY CLEAR. OLD PERICARDITIS

This patient was reëxamined in San Antonio, April 29, 1925. Symptoms of tuberculous disease activity continued absent. Both lungs showed a few scattered dry râles. The percussion flatness in the left axilla and lower back persisted unchanged. No sputum was available for examination. The X-ray confirmed the marked clearing of the process indicated by the physical examination and clinical improvement.

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A COMPARISON OF BREATH-HOLDING TESTS WITH VITAL CAPACITY IN HEALTH AND DISEASE[1]

F. W. WITTICH AND J. A. POLCZAK[1]

Any functional test of physical fitness is interesting to both the observer and the observed. When it is a measure of strength, or physical ability, or fitness, the spirit of contest enters, even among the most handicapped. There is no doubt that the contestants in games centuries ago noticed that the good swimmer or runner must have superior lung capacity.

McMechan (1) calls attention to the fact that as early as 1740 Valsalva showed the influence on the heart-beat of sustained forced expiration in health and disease, and that in 1838 Johannes Mueller observed the effect of prolonged forced inspiration. These fundamental observations showed the close relationship of the respiratory to the circulatory mechanism.

Sabrazes (2) in 1902 collected the literature on the voluntary apneic interval, and observed the normal period of voluntarily holding the breath without either forced inspiration or expiration (that is, between breaths) to be from 20 to 25 seconds, exceptionally, 30 to 35 seconds, and that a forced inspiration prior to holding the breath, or after involuntary expiration during the test, lengthened the period considerably, being from 40 to 50 seconds. Deep-sea divers and expert swimmers have long learned the trick of exhaling a little air at intervals while submerged. Sabrazes also showed the relationship of the apneic pause to pathological states, especially cardiac disease, when the breath-holding capacity fell as low as 5 to 10 seconds and improved after digitalis therapy. He also noted, however, that pulmonary tuberculosis differed from other diseased states in that the apneic period was not so noticeably shortened even in the advanced cases.

Binet and Bourgeois (3) state that the average normal period for holding the breath after a deep inspiration is 50 seconds. The French aviation service set the voluntary apneic interval at 45 seconds as the minimum interval for candidates for high-altitude flying. McMechan states,

[1] Read before the Clinical Section at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 19, 1925.

"Cumulative experience is showing that the breath-holding capacity and apneic pause can be made of the most practical diagnostic and prognostic value in the routine practice of medicine. In simplicity, scope and utility, respiratory tests may be classified with the taking of the temperature, pulse and respiratory rates." They are used at the Berkeley High School, California, as a part of physical functional tests (4).

Bourgeois and his co-workers have made some interesting observations on the influence of maximum voluntary apnea upon the pulmonary rhythm and blood pressure. The lung rhythm, amplitude and ventilation are increased following suspended respiration, and during the pause there is an increase of 10 mm. in the systolic pressure and a lowering of the diastolic of 20 mm. of mercury. The reclining position favors the suspension of the respiration, the average figures being 48 seconds as compared with 36 seconds while sitting and 40 seconds while standing. Bourgeois found that the longest periods of apnea were obtained in the morning, the average being 50 seconds, compared to 40 seconds at night. Even slight exertion in healthy persons, such as bending over and touching the floor four times, appreciably diminished apnea as much as 68 per cent. A cold temperature shortens the pause. There were no clear changes noted between adolescence and old age.

We are now making a series of observations on the apneic pause of cardiac and renal patients after standard exercises, and find that breathlessness is in proportion to the diminished apneic ability and the respiratory pulse-rate response-tests of Cornell (5). Stange (6), of Petrograd, in 1914, using the breath-holding ability test after forced inspiration on many normal patients as well as those suffering from various chronic diseases, recommended it as the best method of determining the patient's cardiac ability to withstand operation under general anesthesia. He also fixed the normal interval at between 40 and 50 seconds.

Fitz-Patrick (7) recently concludes, as the result of breath-holding studies in 871 obstetrical cases of all ages including complications, that the average breath-holding pause (Sabrazes) is 25 seconds; that a reduced interval indicates inquiry; and that the gravid woman with an apneic pause of 15 seconds has an organic lesion, is a poor risk surgically and, if a general anesthetic is given, nitrous oxide-oxygen should be chosen and administered by a trained anesthetist, who is able to determine the individual oxygen need.

W. I. Jones (8), Secretary of the Research Committee, at the request of the National Anesthesia Research Society, made observations on the

oxygen need of his dental patients with complications by means of the breath-holding test, and noted that heart-valve lesions with even early decompensation, diabetics and cases with lowered renal permeability, toxic goitres, anemias and pulmonary tuberculosis, showed markedly lowered breath-holding capacities, and that patients showing a capacity of less than 15 seconds were poor anesthesia risks.

Breath-holding capacity may be compared to breathlessness, and Bie (9) noted that half of the pneumonia patients admitted with a respiration rate over 34 died, and that recovery was usual if the rate was under 34.

It can readily be seen, as McMechan (1) has pointed out, that the apneic pause after deep inspiration and between breaths has its own laws; and he states that "a proper evaluation of the breath-holding tests, as a diagnostic and prognostic indication in abnormal conditions, depends on the realization that the breath-holding capacity and apneic pause involve the oxygen absorption and carbon-dioxide elimination phases of pulmonary respiration, as well as the balance of blood and tissue alkalinity."

According to Yandell Henderson (10) three-fourths of respiration is tissue breathing, and the balance, pulmonary breathing. Whether an anoxemia, acidemia or acapnia, McMechan states, "The breath-holding test is all the more valuable because it represents in its ultimate analysis the response of the body to the oxygen-carbon dioxide balance in the alveolar air, blood and tissues under any and all conditions of health and disease." Mercur (11) has presented a very clear chart showing the relationship between alkali reserve and the breath-holding test in disease.

Mercur's Chart

15 per cent	{Volatile acids Carbon dioxide}	Lungs
85 per cent	{Nonvolatile acids}	Urine and feces

Buffer Salts

20 per cent	80 per cent
A.....B	
Acid radicles	Alkali bases

(Line A B represents all the blood in the body)

By this concept Mercur explains that, in diseased states in which there is deficient elimination, the nonvolatile acids accumulating in the blood will cause the volatile acids to be compressed by the lungs and thereby

increased, so that the breath-holding capacity will be diminished. The difference in time between the two tests (Stange and Sabrazes) is due to the period of time for the absorption of the extra oxygen-containing air on deep inspiration. Oxygen inhalations before the tests markedly increase the apneic pause (Bourgeois).

The determination of the vital capacity and physical fitness, according to Dreyer's (12) directions and formulas, was first applied in this country in 1920 by one of us (13), and the results published in a preliminary report in collaboration with Drs. J. A. Myers and F. L. Jennings. As a test for the individual case, in which the history, physical signs and X-ray have been carefully applied, it seems to bear a distinct relationship to the seriousness of the functional handicap produced by the disease, irrespective of the extent of the lesion. When correlated with the other clinical data it would seem to be of value to determine in a given case of pulmonary tuberculosis the progress of the disease, good or bad.

McMechan (1) notes that the breath-holding test bears a definite and direct ratio to vital capacity, as gauged by the spirometer, so that a normal breath-holding test would naturally suggest a normal vital capacity, and any decrease or increase of the breath-holding interval would indicate a corresponding rise or fall in vital capacity. The vital capacity in cubic centimetres clinically is usually about 100 times the Stange breath-holding interval in seconds for males and the Sabrazes test for females.

We made simultaneous breath-holding and vital-capacity observations on 275 normal males and 63 females. The male group is composed of business men's classes taking gymnasium exercises three to five days a week. These men come from various walks of life, clerks, stenographers, students, business-men, etc., and include many swimmers, so that their capacities would be expected to be exceptionally high, and they proved to be so, the Stange averaging 56.3 seconds and the Sabrazes 36.5. A group of university female students showed a relatively high average for the Stange test, 47.9 seconds, and for the Sabrazes 31.3. Roughly, the average range for normal male adults was from 50 to 60 seconds after forced inspiration, and from 30 to 40 seconds for the apneic pause; and the average range for normal adult females for the former was from 40 to 50 seconds, and for the latter from 25 to 35 seconds (table 1.)

The group of tuberculous men and women showed a very definite corresponding diminution of breath-holding and vital capacity, which diminished notably as the disease advanced. This group represented

TABLE 1

Averages of breath-holding capacities compared with vital capacities of 500 individuals

	BREATH HOLDING TIME (SECONDS)			ACTUAL VITAL CAPACITY	NORMAL (V.C. IN CC.) WEST'S FORMULA	HEIGHT	WEIGHT	AGE
	Number	Stange method (deep inspiration)	Sabrazes method (between breaths)					
				cc.		*cm.*	*lbs.*	
Normal males	275	56.3	36.5	4102	4321	169.8	152.2	29
Normal females	63	47.9	31.3	2923	3219	162.8	126.1	21
Pulmonary tuberculosis (active):								
Males:								
Stage (X-ray) I	4	40 (−28.8%)	28.8 (−21.1%)	4288 (+4.5%)	4247	169.9	151.7	31
Stage (X-ray) II	42	27.3 (−51,5%)	20.8 (−43%)	2619 (−36.2%)	4292	172.9	136.9	36
Stage (X-ray) III	42	22.2 (−60.5%)	15.9 (−56.4%)	2171 (−47.1%)	4315	174.2	135	36
Females:								
Stage (X-ray) I	5	27 (−43.6%)	17.7 (−43.5%)	2019 (−30.9%)	3112	158	94.5	23
Stage (X-ray) II	22	17.1 (−64.3%)	14.7 (−53%)	1717 (−41.3%)	3176	161.7	108.3	28
Stage (X-ray) III	17	17.1 (−64.3%)	13.8 (−55.9%)	1422 (−51.4%)	3125	159.4	119.4	30
Total (all stages):								
Males	88	26.4 (−53.2%)	18.8 (−48.5%)	2481 (−39.5%)	4301	173.3	136.5	30
Females	44	18.2 (−62%)	14.7 (−53%)	1637 (−44%)	3149	160.1	107.2	28
Suspects—females	7	34 (−29%)	18.3 (−41.5%)	2488 (−11.4%)	3359	163	119.5	20
Artificial pneumothorax:								
Males	8	16.8 (−70%)	11.5 (−68.5%)	1919 (−53.2%)	4345	173.8	138.5	28
Females	10	15.1 (−68.5%)	12.9 (−58.4%)	1285 (−56%)	2846	158.1	119.6	25
Bone tuberculosis—males	4	32.9 (−41.6%)	20.6 (−43.4%)	2710 (−33.9%)	4104	114.2	121.8	30
Cardiorenal (various stages of decompensation)								
Males	17	24 (−57.4%)	21.3 (−41.9%)	2330 (43.2%)	4260		146	39
Females	2	23.6 (−50.7%)	21 (−33%)	2875 (−1.3%)	3725		148.5	48

practically all of the adult patients of Nopeming Sanatorium, St. Louis County, Minnesota. All of them were carefully classified, the classification being based upon frequent physical examinations by the staff, and stereoroentgenograms were also taken of all of them. Ninety-nine out of 131 of these patients had positive sputum.

The tests were taken, when at all possible, in the sitting position, the patient remaining quiet for at least five minutes. As suggested by Henderson, the patient was instructed to take a full but not too deep breath, and to hold it with mouth and nostrils closed. The time was noted by means of a split-second stop-watch. The same was done for the Sabrazes test, only there was no forced inspiration, and the patient was instructed to breathe quietly and to hold the breath at the command to do so, an effort always being made to commence the breath-holding between breaths or at the end of quiet expiration and beginning of inspiration. In both tests the nose and lips must be held firmly closed, and no air allowed to enter or leave. There are two normal reflexes noted during the test, namely, the Muset sign or oscillation of the head, and involuntary swallowing which occurred in a number of the cases. Voluntary swallowing of air during the test is forbidden, for it prolongs the interval and the patient in this way gets rid of some carbon dioxide.

The usual precautions were taken for the vital-capacity readings, and at least two and often three observations were made, and the maximum recorded. Sanborn's wet spirometer was used. In estimating the normal vital capacity West's constants (height in centimetres times 20 for females and times 25 for males) were used. But, in order to keep up the comparisons as in the breath-holding tests, the percentages were based upon the average actual vital capacity of our normals instead of the estimated normal vital capacities.

The same statement may be made about breath-holding and vital capacity that Benedict (14) made about basal metabolism: "There is no inflexible standard for normal metabolism for any given age, weight, height and sex, from which all normal individuals never vary." These tests are not to be looked upon as short-cuts to the diagnosis of diseased states. But careful, intelligent application of breath-holding observations to pathological states of the circulatory, respiratory and renal apparatus is a very promising field, and one which the clinician will find worth while. The contrast in breath-holding between the healthy and physically fit group and the tuberculous was striking in most instances.

Many factors influence the tests. The psychical one was notable in

many, as the test was new to the patients, and from two to three observations were often necessary before a satisfactory reading was obtained. Fear of injury to the lungs may have prevented a few from doing their best. We noticed no harmful results, but did not make observations on recent hemorrhage cases. Degenerative changes associated with age, chest deformities, flexibility of the thorax, and factors decreasing the pulmonary volume due to retraction or displacement, such as air or fluid in the thoracic cavity, pleural adhesions, emphysema, abscess or cavity formation, also influenced the breath-holding interval. Also, as in vital capacity, posture, atmospheric conditions, temperature and time of day affected the tests. There, of course, must be differences in the degree of irritability of the respiratory centre. The loss of elasticity of the pulmonary tissue must in part account for the apparent little difference or even reversed discrepancy of some of the far-advanced cases between the Stange and Sabrazes tests. The diminished breath-holding and increased respiratory rate and cyanosis of the advanced consumptive may be due, in part, to the entrance of unaerated blood into the left side of the heart or to the accumulation of the CO_2 in the pulmonary artery (Hirschfelder (15)).

Increased diet and rest, with the laying on of weight, undoubtedly account for discrepancies in breath-holding, as it is well known that during convalescence, with increase in fat, an oxygen-poor tissue, some of the oxygen must be split off as carbon dioxide, with a consequent increase of this substance in the expired air, raising the respiratory quotient. Muscular exercise, which would greatly increase the oxidations of the body and absorption of oxygen to balance this increase of carbon dioxide, is not taken in sufficient amounts, so that there is increased stimulation of the respiratory centre, with a tendency to breathlessness and diminished breath-holding capacity. Cardiac or nephritic complications of the tuberculous also influence the tests.

Peribronchial tuberculosis did not affect the breath-holding or vital capacity to any notable degree. In 18 artificial-pneumothorax cases the breath-holding capacity was influenced by the duration of the collapse, the extent of compression and the condition of the opposite lung. The average Stange was 16.8 seconds and the Sabrazes 11.5 seconds for the males, and 15.1 and 12.9, respectively, for the females.

In 19 cardiorenal hospital cases there was a corresponding decline of the breath-holding interval with the vital capacity, the Stange test averaging 24 and the Sabrazes 21.3 for the men, and 23.6 and 21 for the

women. These were in various stages of decompensation and digitalis therapy, so that frequent observations will have to be made to be of value.

In 4 cases of diabetes mellitus the Stange test approached the Sabrazes, and both showed averages between 15 and 20. In 3 cases of nontuberculous pulmonary suppuration the Stange test averaged 16 seconds and the Sabrazes 21, while the vital-capacity ratio was relatively high. In 4 male cases of bone tuberculosis the Stange test averaged 34 and the Sabrazes 18.3 seconds, the vital capacity being −33 per cent. In this group there was some question of lung involvement. Of a group of 6 suspected cases of pulmonary tuberculosis, according to the Trudeau classification, the average Stange test was 34 seconds and the Sabrazes 18.3 seconds, the vital capacity being −11 per cent. All of these showed a slight corresponding decrease. There was a wide variation in the chronic bronchitis and asthma series depending upon the severity of the disease, the frequency of paroxysms, degree of emphysema, etc., but there was a corresponding diminution of the breath-holding intervals and the vital capacity.

Also, there appears to be a relationship between the pulse-pressure, respiration rate, pulse-rate and hemoglobin index with the breath-holding test, so that the scope, usefulness and simplicity of these tests justify their use along with these others in the routine of clinical diagnosis.

We wish to express our gratitude to Dr. Arthur T. Laird and the Staff of the Nopeming Sanatorium, to Dr. Anna Norris, Director of Physical Education for Women, University of Minnesota, and to Mr. H. B. Monroe, Director of Physical Education, Minneapolis Y.M.C.A., for their generous coöperation and help. Mr. A. S. Levins of the mathematical department also assisted in some of the observations. Throughout we have quoted freely from Dr. McMechan's splendid article which stimulated us to undertake further studies of the subject.

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EXPERIMENTAL INTRATRACHEAL INFECTION WITH THE TUBERCLE BACILLUS

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Although the causative microörganism of tuberculosis has been known and studied during the past forty-three years, the extent of our exact knowledge regarding the pathways of invasion in the more common forms of the disease is slight. It is true that occasional cases are noted in which the primary lesion is situated on the skin or in some other easily recognized position, but these infections are usually self-limited and rarely give rise to a more generalized condition. The two most important portals of entry for tuberculosis appear to be the respiratory and alimentary tracts.

Most cases of tuberculosis, therefore, may be considered as being initiated either by inhalation or by swallowing the bacilli. If the bacteria enter the mouth with food or water, then presumably they pass to the stomach, although it is possible that, temporarily at least, some of them remain in the oral cavity. Inhalation occurs easily as the result of sneezing, coughing or expectorating on the part of consumptives with open pulmonary lesions, or by the entrance of infectious dust stirred up from the floor or pavement. Either by swallowing or by inhalation, therefore, microörganisms reach the pharynx. We still are not certain as to how they reach the lungs.

Concerning the route of *B. tuberculosis* to the site of its pulmonary lesion, certain theories have been advanced. One, which is based upon finding the most advanced lesions in the lungs, states that the microorganisms are aspirated directly into the bronchioles, and that from these points they enter the lung parenchyma through the lymphatics to lodge in certain sites, particularly the peribronchial lymph tissue. Another theory holds that the bacilli gain entrance to the lymphatic system in the upper portion of the respiratory tract. From there they pass directly to the lung by way of the lymphatics. An alternative to this assumption is that the bacterial forms, after traversing the lymphatics to the thoracic duct or to the right lymphatic duct, pass

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through the right side of the heart to enter the lung by way of the pulmonary artery. Another theory which is applicable mainly to those cases of infection resulting from swallowing the microörganism leads us to believe that the intestinal mucosa constitutes the main portal of entry, and that from here the bacilli may pass by way of the mesenteric lymphatics and thoracic duct to the right chambers of the heart, and thence to the lung by the pulmonary artery.

The possible portal of entry through the pharyngeal and tracheal lymphatics may be divided into two parts by lymph drainage. The first, which comprises the tonsils, nasopharynx and pharynx, drains primarily to the superior deep cervical lymph nodes. The second, which includes the lower larynx and trachea, drains through a freely anastomosing plexus in the tracheal submucosa.

Lymph from the tonsils and pharynx may follow several possible paths after reaching the superior deep cervical lymph nodes. Some of it passes to the inferior deep cervical nodes, and then to the thoracic and right lymphatic ducts. The greater portion, however, enters the jugular trunks, and then passes directly to the thoracic and right lymphatic ducts. Then, by way of the heart and pulmonary arteries, this material eventually reaches the lungs. Still another possibility of transfer is offered by the existence of lymphatics connecting the superior deep cervical lymph nodes with the apical pleura. Lymph reaching the pleura by this route would then be carried to the lung by anastomosing lymphatics.

Many early clinicians, notably Klebs (1) and Wassermann (2), basing their conclusions upon clinical evidence, believed that the last-mentioned avenue was the usual pathway of invasion in tuberculosis. Support for this theory has been offered by many subsequent workers, who were able to demonstrate tuberculous lesions in supposedly normal tonsils and in adenoids of persons clinically nontuberculous. Grober (3) in 1905 demonstrated such a pathway experimentally by injecting India ink into the pharyngeal wall of dogs and guinea pigs. At necropsy he was able to show that the particles of ink had traveled down the cervical lymphatics to the apical pleura, and thence directly to the lung parenchyma. Van Zwaluwenburg and Grabfield (4) found that "pleural caps" could be demonstrated by roentgenograms in 93 per cent of their cases of tonsillar tuberculosis. As a result of their work, they believed that the usual path of invasion of *B. tuberculosis* is through the tonsil and cervical lymph nodes to the pleura, and then by direct extension to the lungs.

An abundantly anastomosing lymphatic plexus is found within the tracheal submucosa. It extends from the epiglottis to the bifurcation of the trachea, and here it connects with a similar plexus in the submucosa of each bronchus. Similar anastomosing plexi along the subdivisions of each bronchus connect these with the peribronchial and periarterial lymphatics of the lung. Winternitz, Smith and Robinson (5) (6) have demonstrated this series of lymphatic plexi by India ink. Furthermore, they have shown by gram-stained sections that, after injection of virulent pneumococci into the trachea, the microörganisms enter these lymphatics and then, passing through them, enter the lung. The present contribution concerns itself particularly with the possibility of this last-described route constituting a pathway of invasion for pulmonary tuberculosis.

Before entering upon the more highly specialized discussion of the work of this paper, however, it has seemed wise to incorporate the foregoing description regarding possible lymphatic pathways of invasion of *B. tubercuosis* into a graph. The diagram will clarify the discussion considerably. These various links in this complex series of channels are shown in chart 1.

EXPERIMENTAL WORK

Both bovine and human strains of tubercle bacilli were obtained through the courtesy of Drs. J. A. Traum and E. L. Walker of the University of California, and from the Cutter Laboratories in Berkeley. In addition, five human strains were isolated from tuberculous sputum sent in to the State Board of Health Laboratory for examination.

Isolation of the tubercle bacilli from sputum was accomplished by guinea-pig inoculation. After proving the presence of the microörganisms by acid-fast stains, 2 cc. of the sputum was injected subcutaneously into the inguinal region of guinea pigs. The animals were allowed to live four weeks. They were then killed with ether. Each animal was then fastened to an autopsy board, and the surface of the thorax and abdomen thoroughly wet with cresol disinfectant mixture. A long incision, extending from the lower jaw to the lower part of the abdomen, was then made, and the skin reflected after extending the incision outward toward each leg. A short incision was made along the midline of the abdominal wall with a pair of sterile scissors. Fragments of spleen, 4 to 5 mm. in diameter and containing tubercles, were torn off by a pair of sterile forceps and dropped upon the undisturbed surface of a tube of modified egg-medium. (The formula of this is described

later.) The tubes were sealed, and incubated at 37° for three weeks. If no growth was apparent after this period, the tubes were opened, and the piece of tissue moved around over the surface of the medium by a sterile loop. They were then resealed and returned to the incubator. In all cases growth occurred within a few days after the tissue had been moved.

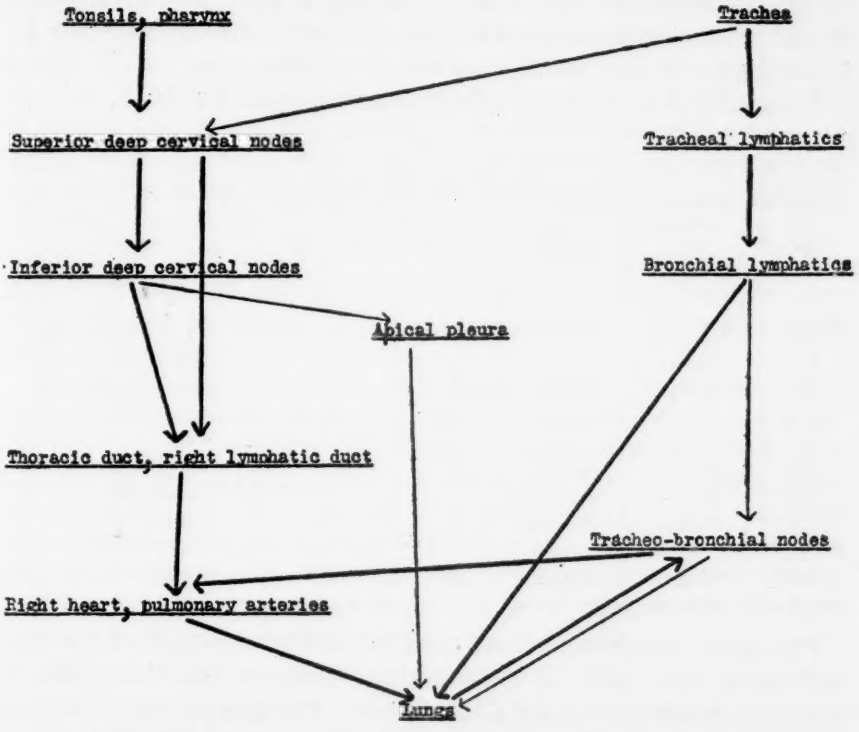

CHART 1. THE LYMPHATIC DRAINAGE OF THE PHARYNX AND TRACHEA

For cultivation, a modified egg-medium was found to give more satisfactory results than Dorset's, Lubenau's, Petroff's or glycerine-agar. This modified medium is made as follows:

(1) Break 12 eggs into a 2-litre flask, and shake until the whites and yolks are thoroughly mixed.

(2) Add 100 cc. meat-infusion broth containing 2 per cent peptone and 1 per cent agar. Filter the mixture through gauze.

(3) Add 5 per cent chemically pure glycerine. Adjust to pH 7.8 electrometrically.

(4) Tube, place in autoclave in slanting position, harden, and sterilize at 15 pounds for 30 minutes. Cool very slowly in order to avoid breaking the medium by the formation of bubbles. After heavy inoculation, growth appears within a few days, and may in favorable cases cover the surface of the slant in one week. Cultures retain their viability for three to four months.

In order to test the virulence, and determine which were most suitable for use, all the strains were injected into guinea pigs. A thick emulsion of each was made in sterile saline solution, and 0.1 cc. of each was injected separately into the inguinal region of guinea pigs.

The results of these animal injections are indicated in table 1.

TABLE 1

Results of virulence tests

STRAIN	TYPE	SOURCE	AMOUNT INJECTED	GUINEA PIG DIED AFTER	LESIONS
			cc.	*days*	
K9	Bovine	Walker	0.1	Killed, 150	Spleen, lymph nodes
B1	Bovine	Cutter	0.1	92	Generalized
B2	Bovine	Cutter	0.1	131	Spleen, lymph nodes
H55	Human	Walker	0.1	128	Generalized
H1	Human	Traum	0.1	Killed, 150	None
HS1	Human	Sputum	0.1	42	Generalized
HS2	Human	Sputum	0.1	79	Generalized
HS3	Human	Sputum	0.1	92	Generalized
HS4	Human	Sputum	0.1	36	Generalized
HS5	Human	Sputum	0.1	84	Generalized

Our main experimental work consisted of the intratracheal injection and subsequent study of the resulting lesions by means of cultures, animal inoculations, smears and sections. The animals used consisted of 2 rabbits and 11 guinea pigs. The rabbits and 7 of the guinea pigs were injected with strain H55, 2 of the guinea pigs with HS3, and 1 guinea pig each with K9 and B2. The last 5 guinea pigs injected received a suspension of the bacilli in India ink instead of saline solution.

All the injections were made intratracheally by the following technique:

The animal was etherized and placed on an animal board. The neck was shaved and the skin painted with iodine. A sterile towel was then placed over the animal in order to avoid infection of the wound as far as possible. A slit cut in this towel provided ample operating room. All of the instruments used were sterilized by boiling water before use. An incision 1½ inches long was made in the midline between the cricoid cartilage and the sternum. After

incision of the superficial cervical fascia, the remainder of the operation was carried out by blunt dissection, with the sternothyroids as landmarks. After exposure of the trachea, we freed it from the surrounding fascia. It was then brought out into the wound by slipping a probe underneath. The point selected for injection was about 1 cm. below the cricoid cartilage. For this, a fine needle and a tuberculin syringe were used. The needle was carefully inserted into the wall of the trachea between the cartilaginous rings, great care being taken to avoid piercing its lumen. A uniform dose of 0.05 cc. of a heavy suspension was used, since it was found impracticable to inject a larger amount if we were to obviate the difficulty of escape of material from the puncture. To lessen this danger, the needle was pushed in as far as possible without penetrating the mucosa, and, after injection, allowed to remain in place a few seconds before withdrawal. The India-ink emulsion used with the last five injections gave visual proof of the absence of leakage. After injection, the probe supporting the trachea was removed and the edges of the wound approximated. The incision was closed either by interrupted sutures or by a running thread. Zinc oxide followed by collodion was applied after painting the wound with iodine, and no drainage was provided. The wound healed promptly by first intention in all cases.

The animals were killed at intervals of 14 to 56 days after operation. They were then placed on an animal board, and the fur wet down with cresol disinfectant. All instruments used at necropsy were sterilized by boiling. The skin was laid back by a long midventral incision, which was extended out laterally along each leg. The abdominal cavity was opened first by a midventral incision and examined for gross lesions. The thorax was then opened by two cuts, one on each side of the sternum. These were extended above the clavicle, using care to avoid wounding the subclavians or innominates, and the whole sternum removed. The trachea was then completely exposed. After examining the thoracic cavity and trachea, the cervical lymph nodes were freed. Portions of the lungs, tracheobronchial and cervical lymph nodes were removed under aseptic precautions, and placed in sterile petri-dishes to be used later for smears, cultures, and animal inoculations. The remainder of the cervical lymph nodes, tracheobronchial lymph nodes, trachea, and portions of the lungs, liver, spleen and kidneys were removed and fixed in Zenker's fluid. Smears were prepared from the cervical and tracheobronchial lymph nodes, as well as from the lungs, liver and kidneys. We attempted to make cultures from the cervical and tracheobronchial lymph nodes in all cases, as well as from the lungs when no gross lesions could be recognized. Animal inoculations were carried through from the pulped tissue in all doubtful cases.

The smears were stained by a modified Ziehl-Neelsen technique as follows:

(1) Stain 2 minutes in boiling carbol fuchsin.
(2) Rinse in water.

(3) Decolorize in 1 per cent hydrochloric acid-acetone.
(4) Rinse in water.
(5) Counterstain 1 minute with saturated aqueous nigrosin.
(6) Wash, dry, examine.

The Zenker-fixed material was imbedded in paraffin and sectioned. Some of the sections were colored with the routine haematoxylin and eosin, or with haematoxylin and erythrosin. Others were stained for acid-fast micro-organisms. The most satisfactory staining method for the tubercle bacilli in tissue was found to be:

(1) Remove paraffin (xylol, alcohol, water).
(2) Hot carbol-fuchsin for 2 to 10 minutes.
(3) Rinse and decolorize to a pale pink in 5 per cent sulphuric acid.
(4) Rinse in acetone for a few seconds.
(5) Counterstain in Loeffler's methylene blue, to which a few drops of saturated alcoholic erythrosin have been added just before use. Stain 30 to 60 seconds.
(6) Absolute alcohol, xylol.
(7) Mount in cedar oil under a coverslip. Balsam causes the stain to fade rapidly. The oil soon hardens sufficiently to hold the coverslip in place. This method gives clear pictures of the histological structure as well as of the bacteria. Moreover, the counterstain is sufficiently light to permit the easy recognition of any ink particles which may be present.

The results obtained from each animal after intratracheal injection are included as follows.

Rabbit 1: Received 0.05 cc. H55 (human). Killed 42 days later. No lesions visible. Smears from cervical nodes, tracheobronchial nodes, lungs, liver, spleen and kidneys negative. Cultures from cervical nodes, tracheobronchial nodes and lungs, negative.

Rabbit 2: Received 0.05 cc. H55 (human). Killed 42 days later. No lesions visible. Smears and cultures all negative.

Guinea pig 1: Received 0.05 cc. H55 (human). Killed 28 days later. *Gross appearance:* Tracheobronchial nodes enlarged; spleen slightly over normal size; cervical nodes, lungs, liver and kidneys negative. *Smears:* Tracheobronchial nodes and spleen positive; cervical nodes, lungs and liver negative. *Cultures:* Tracheobronchial nodes and spleen positive; cervical nodes and lungs negative. *Animal inoculation:* Cervical nodes and lungs positive. *Sections:* Tracheobronchial nodes show moderately advanced lesions with beginning caseation; spleen shows beginning tubercle formation; cervical nodes, lungs and liver negative.

Guinea pig 2: Received 0.05 cc. HS3 (human). Killed 35 days later. *Gross appearance:* Tracheobronchial nodes greatly enlarged; lung shows numerous tubercles; liver pale, has a few large tubercles along its outer margin; cervical nodes slightly enlarged; spleen greatly enlarged,

studded with tubercles. *Smears:* Tracheobronchial nodes, cervical nodes, lungs, liver and spleen positive. *Cultures:* Tracheobronchial and cervical nodes positive; lung cultures contaminated by secondary invaders. *Sections:* Tracheobronchial nodes show advanced tuberculosis with caseation; lungs show scattered tubercles with beginning cavity formation; liver shows extensive perilobular tuberculosis without caseation; spleen with tubercle formation and caseation; kidneys negative.

Guinea pig 3: Received 0.05 cc. H55 (human). Died 4 days later. Autopsy negative. No infection of operative wound. Cause of death undetermined.

Guinea pig 4: Received 0.05 cc. H55 (human). Killed 32 days later. *Gross appearance:* Tracheobronchial nodes enlarged; lungs with a few grayish raised patches; spleen enlarged with small tubercles; liver with few scattered tubercles; cervical nodes slightly enlarged. *Smears:* Tracheobronchial nodes, lungs, spleen and liver positive; cervical nodes positive in 1 out of 4. *Cultures:* Tracheobronchial, cervical nodes and lungs positive. *Sections:* Tracheobronchial nodes involved with moderate caseation; lungs with few patches of tuberculous tissue; spleen moderately involved; liver with scattered miliary tubercles; cervical nodes with slight beginning tuberculosis.

Guinea pig 5: Received 0.05 cc. H55 (human). Killed 20 days later. *Gross appearance:* Tracheobronchial nodes enlarged; spleen enlarged with small tubercles; lungs, liver and kidneys negative. *Smears:* Tracheobronchial nodes and spleen positive; cervical nodes, lungs, liver and kidneys negative. *Cultures:* Tracheobronchial nodes, lungs and spleen positive; cervical nodes negative. *Animal inoculation:* Cervical nodes and lungs positive. *Sections:* Lungs apparently normal, no tuberculous lesions proved; tracheobronchial nodes extensively involved, caseation present; spleen shows tubercles.

Guinea pig 6: Received 0.05 cc. K9 (bovine). Killed 56 days later. *Gross appearance:* All organs negative. *Smears:* Tracheobronchial nodes, cervical nodes, lungs, liver, spleen and kidneys negative. *Cultures:* Tracheobronchial nodes, cervical nodes, lungs and spleen negative. *Animal inoculation:* Tracheobronchial nodes and spleen negative. *Sections:* Tracheobronchial nodes, lungs and spleen negative.

Guinea pig 7: Received 0.05 cc. B2 (bovine in India ink). Killed 37 days later. *Gross appearance:* Spleen shows few large tubercles; all other organs negative. *Smears:* Tracheobronchial nodes, cervical nodes, lungs and liver negative; spleen positive. *Cultures:* Tracheobronchial

nodes, lungs, and liver negative. *Animal inoculation:* Tracheobronchial nodes and lungs negative. *Sections:* Tracheobronchial nodes negative except for scattered ink granules; lungs show lymphoid hyperplasia similar to that found in tuberculosis that has healed by resolution as described by Gardner (7); few scattered ink granules in these lymphoid nodules; liver negative.

Guinea pig 8: Received 0.05 cc. HS3 (human) in India ink. Killed 56 days later. *Gross appearance:* Sinus has formed leading from point of injection to exterior, but not involving lumen of trachea; cervical, tracheobronchial, mesenteric and inguinal nodes enlarged; lung shows scattered tubercles and several small cavities, spleen greatly enlarged and thickly studded with tubercles; liver very pale but without definite tubercles. *Smears:* Exudate from sinus, cervical, tracheobronchial, mesenteric and inguinal nodes, lungs, liver and spleen positive; kidneys negative. *Sections:* Tracheobronchial nodes greatly enlarged and caseous; cervical and mesenteric nodes extensively involved by an acute noncaseating tuberculosis; spleen involved by an acute diffuse tuberculosis between the older discrete tubercles. India-ink particles recognizable in the tracheobronchial nodes and in the lungs.

Guinea pig 9: Received 0.05 cc. H55 (human) in India ink. Killed 21 days later. *Gross appearance:* Tracheobronchial nodes greatly enlarged; lungs show few scattered tubercles; occasional tubercle in liver; spleen enlarged and with scattered tubercles; cervical nodes and kidneys negative. *Smears:* Tracheobronchial and cervical nodes, lungs, liver and spleen positive; kidneys negative. *Cultures:* Tracheobronchial nodes, liver and spleen positive; cervical nodes negative. *Sections:* Tracheobronchial nodes show extensive caseating lesions; lungs have scattered tubercles in the peribronchial lymphoid tissue; small tubercles in the spleen; occasional small miliary tubercles in the liver. India ink present in tracheobronchial nodes and also in tubercles of lung.

Guinea pig 10: Received 0.05 cc. H55 (human) in India ink. Killed 14 days later. *Gross appearance:* Tracheobronchial nodes somewhat enlarged; all other organs normal. *Smears:* Tracheobronchial nodes positive; cervical nodes, lungs, liver and spleen negative. *Cultures:* Tracheobronchial nodes positive; lungs, liver, spleen and cervical nodes negative. *Animal inoculation:* Tracheobronchial nodes and lungs positive; cervical nodes and spleen negative. *Sections:* Tracheobronchial nodes show beginning tuberculosis; lungs, liver, spleen and cervical nodes normal. India ink present in tracheobronchial nodes and in the lungs.

Guinea pig 11: Received 0.05 cc. H55 (human) in India ink. Killed 21 days later. *Gross appearance:* Tracheobronchial nodes enlarged; lung

TABLE 2

Presence of lesions after intratracheal inoculations

ANIMAL	STRAIN	TYPE	KILLED AFTER	TRACHEO-BRONCHIAL NODES	CERVICAL NODES	LUNGS	LIVER	SPLEEN
			days					
R.1	H55	H	42	−	−	−	−	−
R.2	H55	H	42	−	−	−	−	−
G.P.1	H55	H	28	++	−	−	−	+
G.P.2	HS3	H	35	++++	+	+++	++++	++++
G.P.3	H55	H	Died					
G.P.4	H55	H	32	+++	+	+	+	++
G.P.5	H55	H	20	++	−	+?	−	+
G.P.6	K9	B	56	−	−	−	−	−
G.P.7	B2	B	37	−?	−	Healed	−	+++
G.P.8	HS3	H	56	++++	++	++++	++++	++++
G.P.9	H55	H	21	+++	−	+	+	++
G.P.10	H55	H	14	+	−	−	−	−
G.P.11	H55	H	21	++	−	+	−	+

TABLE 3

Result of smears after intratracheal inoculations

ANIMAL	STRAIN	TYPE	KILLED AFTER	TRACHEO-BRONCHIAL NODES	CERVICAL NODES	LUNGS	LIVER	SPLEEN
			days					
R.1	H55	H	42	−	−	−	−	−
R.2	H55	H	42	−	−	−	−	−
G.1	H55	H	28	+	−	−	−	+
G.2	HS3	H	35	+	+	+	+	+
G.3	H55	H	Died					
G.4	H55	H	32	+	+	+	+	+
G.5	H55	H	20	+	−	−	−	+
G.6	K9	B	56	−	−	−	−	−
G.7	B2	B	37	−	−	−	−	+
G.8	HS3	H	56	+	+	+	+	+
G.9	H55	H	21	+	+	+	+	+
G.10	H55	H	14	+	−	−	−	−
G.11	H55	H	21	+	−	+	−	+

shows few scattered grayish areas; spleen somewhat enlarged; cervical nodes, liver and kidneys negative. *Smears:* Tracheobronchial nodes,

lungs, and spleen positive; cervical nodes, liver and kidneys negative. *Cultures:* Tracheobronchial nodes, lungs, and spleen positive; cervical

TABLE 4

Result of cultures after intratracheal inoculations

ANIMAL	STRAIN	TYPE	KILLED AFTER	TRACHEO-BRONCHIAL NODES	CERVICAL NODES	LUNGS	LIVER	SPLEEN
			days					
R.1	H55	H	42	−	−	−		
R.2	H55	H	42	−	−	−		
G.P.1	H55	H	28	+	−	−		+
G.P.2	HS3	H	35	+	+	Contaminated		
G.P.3	H55	H	Died					
G.P.4	H55	H	32	+	+	+		
G.P.5	H55	H	20	+	−	+		+
G.P.6	K9	B	56	−	−	−		−
G.P.7	B2	B	37	−	−	−	−	
G.P.8	HS3	H	56	Contaminated by secondary invaders				
G.P.9	H55	H	21	+	−		+	+
G.P.10	H55	H	14	+	−	−	−	−
G.P.11	H55	H	21	+	−	+		+

TABLE 5

Animal inoculation after intratracheal inoculations

ANIMAL	STRAIN	TYPE	KILLED AFTER	TRACHEO-BRONCHIAL NODES	CERVICAL NODES	LUNGS	LIVER	SPLEEN
			days					
R.1	H55	H	42					
R.2	H55	H	42					
G.P.1	H55	H	28		+	+		
G.P.2	HS3	H	35					
G.P.3	H55	H	Died					
G.P.4	H55	H	32					
G.P.5	H55	H	20		+	+		
G.P.6	K9	B	56	−				−
G.P.7	B2	B	37	−		−		
G.P.8	HS3	H	56					
G.P.9	H55	H	21					
G.P.10	H55	H	14	+	−	+		−
G.P.11	H55	H	21		+			

nodes negative. *Animal inoculation:* Cervical nodes positive. *Sections:* Tracheobronchial nodes show extensive early tuberculosis; lungs

show tubercles in peribronchial lymphoid tissue; spleen shows beginning tuberculosis; cervical nodes, liver and kidneys normal. India ink present in both tracheobronchial lymph nodes and in pulmonary tubercles.

For purposes of presenting a resumé of the results of these experiments, the following tables are included. There is indicated the outcome of each particular set of observations. Table 2 shows the appearance of gross lesions at necropsy. In table 3 are outlined the microscopic findings following smear examinations made for the various organs. Positive

TABLE 6

Summary of all findings after intratracheal inoculations

ANIMAL	STRAIN	TYPE	KILLED AFTER	TRACHEO-BRONCHIAL NODES	CERVICAL NODES	LUNGS	LIVER	SPLEEN
			days					
R.1	H55	H	42	—	—	—	—	—
R.2	H55	H	42	—	—	—	—	—
G.P.1	H55	H	28	L	A	A	—	—
G.P.2	HS3	H	35	L	L	L	L	L
G.P.3	H55	H	Died					
G.P.4	H55	H	32	L	L	L	L	L
G.P.5	H55	H	20	L	A	A	—	L
G.P.6	K9	B	56	—	—	—	—	—
G.P.7	B2	B	37	—	—	Healed	—	L
G.P.8	HS3	H	56	L	L	L	L	L
G.P.9	H55	H	21	L	S	L	L	L
G.P.10	H55	H	14	L	—	A	—	—
G.P.11	H55	H	21	L	A	L	—	L

Key for table 6:

L = gross or microscopic lesions.

S = no gross or microscopic lesions, but smears positive.

A = positive by animal inoculation, negative by all other methods.

and negative cultures are set forth in table 4, while results of animal inoculation from suspected organ macerations will be found in table 5.

A general summary of the work will be found incorporated in table 6 which presents positive findings by the various methods used.

DISCUSSION

On examining the results, the most striking features are the uniform early and extensive involvement of the tracheobronchial lymph nodes, the relatively few lesions in the lungs, the late involvement of the spleen, and the presence of tubercle bacilli, without the formation of gross or

microscopic lesions, in the cervical lymph nodes of all infected animals killed later than 14 days and before the 32nd day. The localized presence of India ink in the pulmonary lesions and in the tracheobronchial nodes of all animals receiving both India ink and virulent microörganisms and in the healed tracheobronchial and pulmonary lesions of guinea pig 7 is also very suggestive. The pulmonary lesions in two cases (guinea pigs 9 and 11) were situated only in the peribronchial lymphoid tissue. This is important, since the presence of primary pulmonary lesions in this place is considered proof of the inhalation theory.

The tracheobronchial and pulmonary alterations seem to show that the primary lesion was either in the lungs or in the tracheobronchial lymph nodes. It is difficult to decide definitely which was first. If in the lungs, the pathway must have been through the tracheal and bronchial lymphatics to the lungs and then by the pulmonary lymphatics to the tracheobronchial lymph nodes. If in the tracheobronchial lymph nodes, the avenue must have been through the tracheal lymphatics to these nodes, then through either the thoracic or right lymphatic duct to the right side of the heart and to the lungs. Lymphatic pathways for either route exist. Hashiba (8), Most (9) and Krause (10) have indicated that the tracheobronchial nodes receive lymph chiefly from the lung and also to some extent from the trachea. The tracheobronchial nodes have no afferents or efferents to the cervical nodes. Mention already has been made of the work of Winternitz, Smith and Robinson (5) (6).

Krause (10) has shown, by sections and animal inoculations, that in less than a week after subcutaneous inoculation of guinea pigs with massive doses of virulent tubercle bacilli, the microörganisms have distributed themselves throughout the body. The pathway of invasion is past the lymphatic barriers into the circulation, through the capillaries of the lungs and into the systemic circulation. The tracheobronchial nodes are rapidly and heavily infected by tubercle bacilli which have been filtered out from the capillaries of the lungs. The early and extensive involvement of the spleen, an organ with no afferent lymphatics, shows that many, if not most, of the bacilli pass through the pulmonary capillaries without lodging. In connection with this point, Rensch and Moore (11) and Krause (12) have called attention to the close correlation in the degree of involvement of the spleen and tracheobronchial lymph nodes after subcutaneous inoculation. They showed that where the microorganisms reach the circulatory system, the spleen and tracheobronchial

lymph nodes show approximately the same degree of involvement. The absence of lesions or their relative scarcity in the lung was attributed to the widely open lymphatic drainage and the scarcity of lymphoid tissue in this organ in the guinea pig.

In all animals showing lesions at necropsy, the oldest processes were in the tracheobronchial lymph nodes. The spleen was always less involved than the tracheobronchial nodes. Also, it appeared to have been invaded later than these nodes. The cervical lymph nodes, although positive by cultures and animal inoculation 20 days after injection, showed definite lesions in only 3 cases. In one of these (guinea pig 8), a discharging tuberculous sinus was present in the neck. In the others (guinea pigs 2 and 4), the involvement was early and slight. These results indicate a late invasion of the systemic circulation, and, since Krause has shown that the microörganisms readily pass the pulmonary capillaries, a late invasion of the pulmonary circulation. Consequently, the results strongly suggest a lymphatic pathway of invasion by way of the tracheal and bronchial lymphatics. The presence of India ink in the pulmonary lesions of the last five animals and the finding of the earliest pulmonary lesions (guinea pigs 9 and 11) in the peribronchial lymphoid tissue supports this view also. The much greater involvement and the presence of India ink in the tracheobronchial nodes may be easily explained by the drainage of the pulmonary lymphatics to these nodes.

This experimental work presupposes the possibility of invasion of the tracheal mucosa without the production of lesion at the point of entry. Numerous workers have shown that this may take place in the tonsils and adenoids. Harbitz (13) demonstrated virulent tubercle bacilli in the cervical and mesenteric lymph nodes of children with no lesions either at the point of entry or in the lymph nodes. Rogers (14) found that tubercle bacilli pass the nasal mucosa of guinea pigs without the formation of lesion at the point of entry. Likewise, Calmette and others (15) have demonstrated this for the pharyngeal and intestinal mucosa. Sanarelli (16) and Grieg (17) showed that cholera vibrios readily enter the tracheal lymphatics. This pathway of invasion may be safely assumed, therefore.

SUMMARY

A series of guinea pigs and rabbits received *B. tuberculosis* by intratracheal injection. In certain instances, the injected microörganisms were suspended in India ink. Subsequently, some of these experimental

animals developed pulmonary tuberculosis. Microscopic examination revealed acid-fast bacilli and ink particles in the same tissue field. An available lymphatic pathway to the lung from the site of injection has already been demonstrated. We believe, therefore, that resultant pulmonary infection in the animal following the insertion of *B. tuberculosis* within the wall of the trachea is developed from microörganisms traversing the tracheal lymphatic pathway.

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OXIDATION AND REDUCTION AT THE SITE OF A TUBERCULOUS LESION[1]

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In another paper there has been described the morphological changes that occur in the monocyte and the tubercle bacillus as a result of their commensal relations in the animal body (1). These may be summarized again as follows: The monocyte swells, loses its outlines, fuses, alters in staining reactions of nucleus and protoplasm, increases in amitotic division, becomes fixed in lodging, segregates fat, dies, and furnishes part of the final caseous mass of the progressing tubercle. Tubercle bacilli grow singly instead of in clumps and skeins: they become granular, segregate spots of different tinctorial value, and form granules that are no longer acid-fast. They may even fail to stain by our present staining methods, even though they are still present in the caseous substance, as can be shown by animal inoculation. In the same paper it was suggested that the monocyte was the natural host of this microorganism; that the latter may be either parasite, symbiote or commensal, in relation to this host, but always parasitic when disease results; and that, depending upon its adaptability to this host, its virulence and mutant characteristics are established.

The first monocytes of a progressing tubercle usually die, as do many of their fellows, and the bacilli which they carried are either left embedded in the caseous mass or become residents in new monocytic hosts. It is in the cells at the periphery of a tubercle that the commensal changes in the two partners are apparent.

The other physical responses of the tuberculous infection, such as the primary exudate of plasma, which later coagulates and forms reticulin, and the collection of lymphocytes are not considered in this paper. Nor are the chemical changes that occur in the body fluids and other cells of the body considered. The latter changes, as is apparent in the

[1] Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

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tuberculin reaction, are very pronounced, and must be considered in any attempt to present a complete picture of the tuberculous process, but they are not at all peculiar to infection with the tubercle bacillus and will only be understood as the fundamental laws of living cells in their congregate relations are more accurately known.

We have, therefore, in the following studies asked ourselves whether any of these changes in the two cells can be demonstrated to be modifications of fundamental life processes. The present paper deals with the phenomena of oxidation and reduction in this area of local change due to tuberculous infection.

Of the mechanisms of oxidation and reduction in living cells we are still fairly ignorant. The one mechanism that has been isolated is the sulphydril-double-sulphide transformation group discovered by F. Gowland Hopkins (2) and by him called *glutathione*. This substance, a combination of cystein and glutamic acid, has the formula

$$\mathrm{COOH \cdot CH(NH_2) \cdot CH_2 \cdot CH_2 \cdot CO \cdot NH \cdot \underset{\displaystyle \mathrm{CH_2SH}}{\mathrm{CH}} \cdot COOH}$$

Oxidation and reduction of this substance by tissue systems involve the thio group alone and the strictly reversible change

$$\mathrm{-SH \cdot HS- \rightleftarrows -S-S- + H_2}$$

Animal tissues contain a substance capable of activating this change in glutathione. Glutathione is found in fresh extracts of all animal cells and most plant cells. It is autoxidizable, and the change is reversible. It acts as a hydrogen acceptor at one stage and a hydrogen donator at another stage.

This mechanism in its active form is not found in the tubercle bacillus. One of us (Sullivan) has shown, by careful analysis of tubercle bacilli grown on glycerine-agar and on Long's synthetic medium (3), that there is present no active glutathione, no cystein and no oxidized glutathione in this microörganism.[3] There is however a small amount of material reacting like cystine. Checked against control solutions of pure cystine this amounts to between 200 and 300 parts per million (4). Dr. Johnson tells us that in his work he finds no evidence of sulphur

[3] The moist bacilli grown on Long's medium were furnished by Parke, Davis & Co., and the air-dried bacilli by H. K. Mulford & Co.

in the protein fraction of the tubercle bacillus, and Johnson and Coghill (5) have found cystine present in amounts too small to be estimated in analysis on material from tubercle bacilli from the same source.[3] Dr. Sullivan by color reactions finds distinct traces of sulphur in the whole bacillus in the cystine fraction. The details of this study will appear in the published report of this work.

The premise of this paper consists, then, in this observation that, in tuberculous infection, there exists a commensalism which involves two oxidation-reduction mechanisms; the monocyte operating in part, at least, by the sulphydril-disulphide mechanism, and the tubercle bacillus, which contains no machinery of this sort, by some other process. In other words, we have two competing systems for available labile hydrogen, in the symbiotic relations of these two living organisms, the tubercle bacillus and the monocyte.

What mechanism the tubercle bacillus uses cannot be stated although one is tempted to suggest that the unsaturated fatty acids play a rôle, possibly those of the linoleic series. Possibly, in the light of the findings of Johnson, Brown and Coghill (6) on the high percentage of hexose bases, it may be found that glyoxalase is an operating mechanism, as was suggested by the important work of Dakin and Dudley (7). But for the moment the fact that two different mechanisms exist in these two cells is the immediate concern and, that it may be one of the great factors in the morphological changes in host and parasite which are described above, seems more than likely when the nature of some of these changes are considered, especially the segregation of fat and the different pH values in the different cell areas referred to below. Many examples of the complete change of events by combining two oxidation-reduction mechanisms in the test-tube are known, and the radical revolution that may be caused in such a system by the simple introduction of an inorganic catalyst adds presumptive evidence to the correctness of this suggestion.

Other evidences of a disturbance of the oxidation and reduction process in the tuberculous tissues are not wanting. We will mention here only four that have been a part of our own study: (1) the nitroprusside test, (2) injections of methylene blue, (3) injection of glutathione into the tissues infected with tuberculosis, and (4) tissue colors resulting from the injection of indicators having a color range varying with the pH of the medium.

THE NITROPRUSSIDE TEST

Normal cellular tissues all give the nitroprusside reaction (8). This is a test for the presence of the sulphydril groups, as they occur in cystein and reduced glutathione. These compounds give an amethyst color in solutions of sodium nitroprusside in the presence of ammonium hydroxide. Of all the tissues the most actively proliferating give the strongest reactions; thus it has been found that the testicle is one of the most striking tissues both in rapidity of development and in the intensity of the color produced. Dr. Smith has followed this reaction in normal and tuberculous testicles, and finds a diminishing reaction in direct relation to the progress of the disease. This test, as we have applied it, gives no evidence of the contrast of reaction between the caseous centre and the surrounding abnormal and normal tissues, but it shows that, as caseation increases in amount, the nitroprusside reaction diminishes in intensity, and this may be interpreted as meaning that in the mass there is a diminution of the active sulphydril-disulphide mechanism in the whole area infected with tuberculosis, growing less as the tuberculosis increases in severity.

INJECTIONS OF METHYLENE BLUE

A good deal of the work with methylene blue is not reliable. Even such careful work as that of DeWitt (9) is not free from criticism. DeWitt complicated her method by exposing her tissues to solutions of ammonium molybdate. Normal tissues unimpregnated with methylene blue and without tuberculous infection readily reduce this compound to a blue oxide, and the surface of the tissue block so exposed becomes covered with a deposit of irregular intensity very similar in color to methylene blue and this is often denser over the tuberculous mass. Methylene blue alone, given in the doses recommended by DeWitt, did not give us color around the tubercle on exposure to air. On the other hand, 30 cc. of 10 per cent methylene-blue solution, injected intravenously into a rabbit with tuberculosis of the lungs, did give us a definite blue ring about the tubercles after exposure to air. The animal was chloroformed while still blue and autopsied immediately. Whether the methylene blue is not quite reduced to its leucobase by the diminution in the glutathione mechanism in the region, and therefore oxidizes more readily than that in the normal tissues on exposure to air; or, whether there is a complete change in the chemical process between a

portion of the tuberculous area and the normal tissue, we do not know. A strong oxidation agent like chlorine water will not restore the blue color to the surrounding tissues, and the caseous centre remains white throughout. This observation is offered here only as evidence that there is a definite modification in the oxidation-reduction process at the site of a tuberculous lesion. Fresh tissues reduce methylene blue to its leucobase, and these are not reoxidized if the tissues are uninjured and exposed to air or to stronger oxidizing agents. The presence of the tubercle bacilli in some way changes this process, either by competing for the available labile hydrogen or by injury to the normal activity of the collected monocytes in which they grow.

GLUTATHIONE INJECTIONS

We have obtained a striking picture by injecting glutathione into a testicle recently infected with tubercle bacilli. A series of 9 male rabbits were given 2 mgm. of H37 (Saranac strain of tubercle bacilli) in normal salt solution in each testicle on February 25. On February 26, 27, 28, and March 2, they were given daily, into the substance of the right testicle, 8 mgm. of glutathione in distilled water. On March 5 the animals were chloroformed, and both testicles were removed. The left testicle in all of them appeared normal. Microscopic study revealed the customary early tuberculous changes. In 6 of the 9 rabbits the right testicle was studded with cheesy-looking masses, very similar in gross appearance to those lesions which we described a year ago as resulting from the injection of what we called endothelial extract (10). Microscopically, the lesions resulting from glutathione are very different. In the lesions caused by endothelial extract epithelioid cells formed the major portion of the picture. In the latter, those formed by injection of glutathione, the lesions are small areas of necrosis. There is no increase in epithelioid cells and no evidence of inflammation due to any pus-producing infection. Glutathione alone in a control series caused no lesion. With another preparation of glutathione we were not so successful in producing the lesions, but the method of preparation of the glutathione we were using was different. Evidently a combination of tubercle bacilli and glutathione in the tissues was responsible for this picture.

This work is still in progress, but owing to the difficulty of preparing glutathione it cannot proceed very rapidly. The glutathione was prepared by Sullivan following the method of Hopkins.

An interpretation of the results without more work would be impossible at this time. This much, however, may be said: that under some conditions the introduction to an area of tuberculous infection of an excess of such an oxidation-reduction mechanism as glutathione may grossly modify the process of the infection.

TISSUE REACTIONS

W. Mansfield Clark (11) and others have shown that the hydrion concentration of a medium is quite as important a factor as the equilibrium of oxidant and reductant in any system of oxidation and reduction. Rous (12) has shown, using color indicators recommended by Clark (13), that the granules of the macrophage, which is the same cell that we have called in this paper the monocyte, have a hydrion concentration ranging as high as pH 3 or even less. This is higher than the other cells studied. This cell has a longer life than a polymorphonuclear phagocyte and a more pronounced acid reaction gauged by its content of acidophilic granules. The surrounding fluids, serum and plasma, in which these macrophages live and move, have an alkalinity as low as pH 8.4. Tubercle bacilli in test-tube cultures grow best at pH 6–8. The pH of the medium, in which tubercle bacilli in the test-tube grow, varies with the age of the growth and with the strain of the microörganism from pH 6.4 to pH 8, but it is rarely constant.

In some preliminary work on tuberculous tissues (fresh rabbits' lungs from animals injected intravenously with 2 mgm. of tubercle bacilli of our bovine strain 435 two months before autopsy) we found that frozen sections placed in solutions of Clark indicators, within thirty minutes of the time when the animal was alive, gave definite differences between the reaction of the centre of the tubercle and the surrounding, apparently normal, lung tissue; and that the color shaded from the centre to the periphery of the tubercle. The frozen sections were placed in the following indicators, with the pH range put in brackets after each indicator:

1. Thymol blue..... (pH 1.2–2.8)	5. Brom cresol purple..... (pH 5.4–7.0)
2. Brom phenol blue..... (pH 3.0–4.6)	6. Brom thymol blue..... (pH 8.2–98.)
3. Brom cresol green..... (pH 4.0–6.0)	7. Phenol red..... (pH 6.6–8.2)
B. Methylene red..... (pH 4.4–6.0)	8. Thymol blue..... (pH 8.2–9.8)

It was found that in the following indicators there was a distinct difference between the centre of tubercle and surrounding tissues:

brom phenol blue, brom cresol green and methylene red. The resulting color indicated the reaction of the centre of the tuberculous area to be in the neighborhood of pH 4. The surrounding tissues were all alkaline, giving a definite color picture ranging between pH 7 and pH 8.

This work also is in progress, but it is open to objection, especially since the frozen sections were cut with CO_2 and were exposed to air, both of which may modify the end-reaction picture. But, because a difference was found between the caseous centre and the surrounding tissues, it is mentioned here, as the difference, if true in life, would have a distinct bearing on the rate and character of the oxidation-reduction process of the area involved.

CONCLUSION

A series of observations is recorded in support of the suggestion that, since, in the conjunction of the tubercle bacillus and the monocyte in their commensal existence, there are operating two different oxidation and reduction mechanisms—the monocyte in common with other body cells containing glutathione, the tubercle bacillus containing no glutathione, there is a possibility, in the competition of these two mechanisms for available labile hydrogen, of explaining the observed alteration in the structure of both organisms.

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THE ACTION OF UNSATURATED FATTY ACIDS ON TUBERCLE BACILLI[1]

CHARLES H. BOISSEVAIN

In the search for a substance which might be used to kill tubercle bacilli or inhibit their growth in the body, many observers have investigated various oils and fatty acids, notably codliver and chaulmoogra oils and their derivatives. The results have not been consistently encouraging, and these substances retain only a minor or insignificant place in therapy.

In 1923 McJunkin (1) reported experiments, in which living tubercle bacilli were dehydrated for a few minutes with alcohol or acetone and then mixed with oleic acid (U. S. P. Merck) containing a little moisture, and incubated at 37°C. After several days most of the bacilli were found to be non-acid-fast, and after six or eight weeks the volume of the bacillary mass had greatly diminished. If dehydration was prolonged, there was little or no effect from the subsequent treatment with oleic acid. The effect was not produced with less concentrated solutions and emulsions. Heat-killed cultures resisted the action of the acid in full strength.

In a later communication, McJunkin (2) published similar results with acetone and olive oil, and with acetone and liquid petrolatum. Guinea-pig inoculations with bacilli which had been treated with oleic acid or olive oil, after dehydration, produced no disease, and it seemed reasonably certain that the bacilli were dead.

Our results with oleic acid agree with McJunkin's as regards loss of acid-fastness and virulence. However, we have found the preliminary dehydration to be unnecessary, and have brought about loss of virulence by the treatment of tubercle bacilli with emulsions of fatty acids, and loss of acid-fastness in bacilli previously killed in the autoclave at 110°C.

[1] From the laboratories of the Colorado Foundation for Research in Tuberculosis at Colorado College, Colorado Springs.

REPORT OF EXPERIMENTS

Our studies have dealt mainly with the following members of the aliphatic series:

Linolenic acid ($C_{18}H_{30}O_2$), prepared from linseed oil according to the method described by Rollet (3). It showed an iodine number 220, corresponding to 50 per cent alpha and 50 per cent beta-linolenic acid.

Linolic acid ($C_{18}H_{32}O_2$), prepared from poppy oil according to Rollet (4), with an iodine number of 165, corresponding to 90 per cent linolic acid.

Oleic acid ($C_{18}H_{34}O_2$) from Mallinckrodt.

Stearic acid ($C_{18}H_{36}O_2$) from the Denver Fire Clay Co.

All the acids used were carefully washed with distilled water, until this reacted neutral to litmus.

The tubercle bacilli used were of a human strain (*Gray Z*), recovered from sputum and now under cultivation for several years. It kills guinea pigs in about three months after the subcutaneous inoculation of fairly large doses.

EFFECT ON ACID-FASTNESS

The results of experiments on loss of acid-fastness after treatment with the fatty acids and some other substances are given in table 1.

The power of the fatty acids to render tubercle bacilli non-acid-fast, without preliminary dehydration, increases in the following order: stearic (none), oleic, linolic, linolenic. All of these acids have a long carbon chain (18C) and a $-COOH$ group. Stearic acid has no double bonds, oleic has one, linolic two, and linolenic three. Among the other substances studied, propionic acid, which has a three-atom carbon chain, a $-COOH$ group, and no double bonds, produced a slight loss of acid-fastness; valeric acid, with a 5C chain, a $-COOH$ group, and no double bonds, produced a considerable loss. The effect of these acids, in the absence of preliminary dehydration of the bacilli, is probably enhanced by their ready solubility in water, a point to be discussed later.

No effect was produced by acetic acid (2C chain, $-COOH$ group, no double bond), nor by 10 per cent solutions of the sodium soaps of stearic, oleic, linolic, and linolenic acids (no $-COOH$ groups). Amylene, with a 5C chain, and a double bond but no $-COOH$ group, pro-

TABLE 1

SIX WEEKS' BROTH CULTURE OF TUBERCLE BACILLI IN	TEMPERATURE	DURATION	LOSS OF ACID-FASTNESS
	°C.		
Linolenic acid	37	24 hours	0
	56	30 minutes	+++
	130	5 seconds	+++
Linolic acid	37	24 hours	0
	56	30 minutes	+++
	130	5 seconds	+++
Oleic acid	37	24 hours	0
	56	30 minutes	++
	130	5 minutes	+++
Stearic acid	37	24 hours	0
	56	30 minutes	0
	130	5 minutes	0
Vaseline	37	24 hours	0
	56	30 minutes	0
	130	5 minutes	0
Linseed oil	37	24 hours	0
	56	30 minutes	0
	120	5 minutes	0
No preliminary treatment			
Linolenic Na soap, solution, 10 per cent	94	15 minutes	0
Linolic Na soap, solution, 10 per cent	94	15 minutes	0
Oleic Na soap solution, 10 per cent	94	15 minutes	0
Stearic Na soap solution, 10 per cent	94	15 minutes	0
Valeric acid	37	24 hours	+
	56	30 minutes	++
	130	5 minutes	+++
Propionic acid	37	24 hours	0
	56	30 minutes	+
	130	5 minutes	++
Acetic acid	37	24 hours	0
	56	30 minutes	0
	100	5 minutes	0
Amylene	20	24 hours	0
After washing in acetone			
Stearic acid	130	5 minutes	+++
Linseed oil	120	5 minutes	++
Vaseline	200	5 minutes	++

0 = no loss of acid-fastness. ++ = loss of acid-fastness by many bacilli.
+ = loss of acid-fastness by a few bacilli. +++ = loss of acid-fastness by all bacilli.

duced no loss of acid-fastness; owing to its low boiling point it was tried only at 20°C. Vaseline, a mixture of hydrocarbons of about 18C, had no effect on bacilli which had not been dehydrated; neither had linseed oil, which is largely composed of fat (linolenic glyceride) with an 18C chain and three double bonds.

Bacilli, which had first been dehydrated by washing with acetone, were rendered partially non-acid-fast by brief treatment at high temperatures (120°–200°C.) with vaseline and linseed oil, and entirely so by stearic acid. These substances are extremely insoluble in water and cannot penetrate a coating of moisture.

It appears, then, that the power of the unsaturated fatty acids to destroy the acid-fastness of moist tubercle bacilli, which presumably depends on ability to penetrate them and dissolve their fatty components, is correlated with four properties; namely, a long carbon chain, a $-COOH$ group, double bonds, and solubility in water.

In order to determine whether there is a parallelism between unsaturation (double bonds) and solubility in water, the following experiment was performed: 5 cc. of N/50 sodium soaps of stearic, oleic, linolic, and linolenic acids were mixed with 5 cc. of N/50 HCl, giving 10 cc. of N/100 fatty-acid emulsions. Higher dilutions were made in the same way, compared in the nephelometer, and the readings plotted. Each resulting curve showed a discontinuity; that for stearic acid showed two, one corresponding to that of oleic acid, and perhaps due to a mixture of palmitic acid, the other at a higher dilution. The points of discontinuity indicate the following solubilities, increasing with the number of double bonds:

Stearic acid (no double bond)	N/500,000
Oleic acid (one double bond)	N/50,000
Linolic acid (two double bonds)	N/10,000
Linolenic acid (three double bonds)	N/5,000

EFFECT ON VIRULENCE AND VIABILITY

Preliminary to the study of the effect of the fatty acids on the virulence and viability of the tubercle bacillus, the following experiments were performed: N/1000 emulsions of the fatty acids were mixed with a suspension of tubercle bacilli and incubated over night. The next day they were centrifugated, and compared in the nephelometer with control emulsions of fatty acids and with the centrifugated suspensions of tubercle bacilli. In the case of oleic and stearic acids there

was no evidence of absorption; in the case of linolic acid 0.1 cc. of the bacillary suspension produced a considerable reduction in the opacity of a N/1000 emulsion, while 0.01 cc. of the suspension had a similar effect on a N/200 emulsion of linolenic acid. That is, the absorption of these acids by the tubercle bacillus increases with the solubility in water, which in turn increases with the number of double bonds.

To determine the effect on virulence, 20 mgm. (wet weight) of tubercle bacilli were mixed with 2 cc. of N/10 emulsions of oleic, linolic, and linolenic acids, and with 2 cc. of N/10 solutions of stearic, oleic, linolic, and linolenic sodium soaps, and incubated 24 hours. Stearic acid was not used, as it will not remain emulsified in this concentration. After incubation, the bacilli were injected into guinea pigs. Those which had been treated with the fatty acids produced no lesions, so far as could be seen at autopsy after four months. Those which had been treated with soaps produced the usual fatal disease, the guinea pigs dying in about the same time as controls, that is, in about three months. After three-hour or one-hour incubation with the same substances, the results were the same.

Cultures on Dorset's egg medium, after one-hour incubation with the fatty acids and subsequent washing in weakly alkaline normal salt solution, gave no growth in the case of linolic and linolenic acids, and some growth after six weeks in the case of oleic acid.

EFFECT ON EXPERIMENTAL TUBERCULOSIS

Three guinea pigs were inoculated with a culture of human tubercle bacilli of standard virulence, and were then given 9 doses of 0.5 cc. linolenic acid subcutaneously at 2-day intervals. Linolenic acid was selected, both because of its powerful effect on the bacillus *in vitro*, and because it can readily be assimilated as fat into the tissues of the living mammalian body. At the end of this time the tuberculin skin test was as strongly positive as that in control animals. All died in about three months.

Thus, once more a substance which is very effective against the tubercle bacillus *in vitro* was found to have no effect *in vivo*. In this instance failure may have been due to the saponification of the fatty acids by the buffer salts of the blood and tissues. To test this point, emulsions of linolenic acid were made in buffer solutions, ranging from pH 8.4 to pH 5.5, and compared in the nephelometer with an emul-

sion of pH 12. The minimum opacity occurred at pH 7, indicating that at this pH the acid is in solution as soap. The greater opacity of the solutions on the alkaline side of neutrality is probably due to the salting out of the soaps. Soaps, as already stated, have no effect on the tubercle bacillus.

CONCLUSIONS

1. Tubercle bacilli *in vitro* are made to lose acid-fastness and virulence, and are apparently killed, by treatment with certain fatty acids of the aliphatic series, in full strength and in emulsion. Preliminary dehydration is not necessary.

2. The effect seems to depend on the presence of a long carbon chain (5C) and a $-COOH$ group, and to increase with the number of double bonds (degree of unsaturation), and the corresponding increase in solubility and power of being absorbed by the tubercle bacillus.

3. Linolenic acid, the most effective fatty acid *in vitro*, has no effect on the course of tuberculosis in the guinea pig. This may be due to the fact that in buffer solutions of pH 7 the acid is converted into soap, which is harmless to the tubercle bacillus.

The author wishes to express his indebtedness to Dr. G. B. Webb and Dr. C. T. Ryder for their coöperation.

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A METHOD FOR OBTAINING SINGLE COLONIES OF TUBERCLE BACILLI[1]

CHARLES H. BOISSEVAIN

For the study of variation of virulence, or any other biological property of bacteria, it is important to use cultures derived from a single organism. Ordinary cultures, made by planting millions of bacilli and securing a massive confluent growth, are likely to contain a mixture of strains which may differ widely from one another. With organisms which grow vigorously on artificial media it is easy, by simple planting, to get colonies, which we are reasonably sure have grown from one individual; or we may use the Barber technique (1) and be quite certain; but with the tubercle bacillus such methods usually give no growth. In the hope of finding a satisfactory method the following procedures have been tried, with encouraging results:

A: A rabbit is bled from the carotid artery and the blood received into paraffined centrifuge tubes packed in ice, and centrifugated at once. A rather light suspension of tubercle bacilli in 1.5 per cent sodium-chloride solution is prepared, filtered through sterile filter paper, and centrifugated for a few minutes. One drop of the supernatant suspension is mixed with 1 cc. of the rabbit plasma in a sterile tube, stoppered with paraffined cotton, slanted to form a thin layer, and left to coagulate in the incubator. Coagulation occurs in about ten minutes, and after that the tubes should be left lying on their sides. In an upright position all the serum is pressed out of the coagulum and collects at the bottom of the tube. In about two weeks many small discrete colonies can be seen *in* the plasma, but not on the surface. These can be transplanted or used at once for inoculation. This confirms an observation of A. E. Wright (4) on the multiplication of tubercle bacilli in capillary tubes filled with plasma.

B: Rabbit serum, with the addition of an equal volume of 3 per cent agar in distilled water at 50°C., but without peptone, salt, or glycerine, is substituted for the plasma. With this method, the results have

[1] From the laboratories of the Colorado Foundation for Research in Tuberculosis at Colorado College, Colorado Springs.

varied. Serum from some rabbits has yielded a few colonies; serum from others has yielded none; in no instance has the growth been as abundant as with plasma.

C: Freshly ground rabbit liver is added to the rabbit serum of method *B*, in the proportion of about 1 to 20.

In this medium the tubercle bacilli grow as well as in plasma. The liver of a tuberculous guinea pig may be used, and primary growth be secured in the form of single colonies. There would, however, be more doubt of the colonies having developed from a single bacillus than when a light filtered and centrifugated bacillary suspension is used.

D: In "hormone agar" (2) (3) plus rabbit serum, in equal parts, colonies develop abundantly. If the serum is omitted, a few colonies sometimes appear. Experiments are now in progress to ascertain what substance present in tissue and in plasma is so favorable to the growth of the tubercle bacillus.

SUMMARY

The method of Koch for obtaining single colonies has been adapted to Koch's bacillus.

The author wishes to express his indebtedness to Dr. G. B. Webb and Dr. C. T. Ryder for their coöperation.

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AN ATTEMPT TO ESTABLISH TISSUE-SPECIFIC STRAINS OF TUBERCLE BACILLI[1]

LYDIA M. DEWITT AND H. GIDEON WELLS

Tuberculous infections at times exhibit striking examples of what seems to be selective tissue affinity; for example, the cases of widespread invasion of the lymph nodes without involvement of other tissues, or the extensive fibrocaseous tuberculosis of both adrenals, with little or no gross tuberculosis elsewhere, so often seen in Addison's disease. In view of these and similar manifestations of tissue selectivity in human tuberculosis, it was thought that it might be possible to secure strains of tubercle bacilli with a selective affinity for specific tissues in experimental animals, which strains might well have some value in certain lines of investigation. Experiments with this end in view have, therefore, been carried on for several years, but with results so persistently negative that we have at last decided to abandon them. As these experiments have involved much work and would require so long a time for duplication, it is thought that a brief statement should be placed on record for the benefit of others interested in the problem of tissue specificity in infections.

The experiments were conducted as follows: A guinea pig was inoculated with a virulent strain of human tubercle bacilli, known as *Miller's strain*, and was killed when a generalized infection had developed. The involved lymph nodes were collected, ground with quartz sand and saline solution in a mortar, strained through fine wire gauze, and three guinea pigs were inoculated subcutaneously with varying amounts of the fluid. Another set of three animals was inoculated with emulsions prepared in a similar way from the spleen, a third set was inoculated with liver emulsion, and a fourth set with lung emulsion. These four sets of animals were kept separate, and designated respectively as the lymph-node, spleen, liver and lung series. Whenever an animal of one of these series died with demonstrable tuberculosis, one of the surviving members of the same set was killed with illuminating gas, and the tissue designated

[1] From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago.

for this series was removed aseptically and used for inoculating three more guinea pigs. The surviving member was permitted to live as long as possible, and when it died an autopsy was performed upon it, as also had been done with the other two members of this set. In this way we developed series of animals in which for passage after passage the inoculation was always made with the tubercle bacilli that were growing in the same sort of tissue. It was thought that perhaps in this way strains of tubercle bacilli might be secured which would exhibit a greater affinity for, or power for growth in, the four selected tissues. This seemed a more reasonable method than the one sometimes tried, of growing the bacteria in artificial media containing the tissues or extracts therefrom, since in the living animal the microörganisms with the greatest capacity to grow in the selected tissue would presumably be the ones that could best survive the defensive mechanism of the host.

These experiments have been carried on for nearly ten years. At times death from intercurrent infections or accidents has destroyed a set of animals before the tuberculosis had developed to a degree permitting of successful inoculation from the desired tissue. In such cases a new series was started. With the lymph nodes a series of 35 generations of inoculations was accomplished; with the liver the longest series was 29 generations; with the spleen 38 generations were observed; but with the lung at most only 9 generations were successfully inoculated. At no stage of this work, even in the longest series, was there any evidence of tissue selection on the part of the infection. The animals showed always the usual distribution of lesions in the lymph nodes, liver, spleen and lungs, with no more than the common variations seen in tuberculous animals, the lesions predominating in the lymph nodes and spleen just as much in the liver and lung series as in the lymph-node and spleen series. We recognize various reasons why such a method as the one we selected might fail to develop tissue-specific strains of tubercle bacilli, and merely record here that in our hands it has failed so completely that we do not consider it a profitable venture to carry farther.

A COMPARISON OF THE DEATH-RATES FROM TUBERCULOSIS IN 23 LARGE CITIES OF THE UNITED STATES

HOWARD WHIPPLE GREEN[1]

Comparisons of the tuberculosis mortality in the large cities of the United States are frequently made without heed to the specific death-rates for color and age, although it is well recognized that the death-rate from tuberculosis varies considerably with age-group and color.

In the preparation of the accompanying table, the age-group 20–29 years has been selected because of the high death-rate at this particular period. The white and colored rates have been calculated for the total population and for this specific group. The white death-rate per 100,000 for all ages varies fom 176 to 67 while the colored death-rate for all ages varies from 504 to 162. For the 20–29 year age-group there is greater variation, that is, from 223 to 52 for the white, and from 754 to 262 for the colored.

Los Angeles, California, has the highest white death-rate for all ages and for the 20–29 year age-group, and for the colored it ranks fourth for all ages and second for the 20–29 year age-group, while Cincinnati, Ohio, has the highest colored death-rate for all ages and for the 20–29 year age-group as well.

Birmingham, Alabama, has the lowest white death-rate for all ages and for the 20–29 year age-group, and for the colored it ranks fourteenth for all ages and eleventh for the 20–29 year age-group, while Atlanta, Georgia, has the lowest colored death-rates.

The age-specific death-rates for the white and colored differ greatly in such cities as Cleveland, which ranks eleventh for the white and fifth for the colored, the rates being 148 and 550, or a colored rate 3.7 times the white; Louisville, Kentucky, which ranks second and twenty-second for the white and colored and Detroit which ranks fifteenth for the white and nineteenth for the colored, and Dallas, twenty-second and fourth, the rates being 56 and 610, the colored rate being nearly eleven times the white.

[1] Director, Bureau of Statistics and Research, Cleveland Health Council, Cleveland, Ohio.

COMPARISON OF DEATH-RATES FROM TUBERCULOSIS IN 25 DIFFERENT CITIES IN THE UNITED STATES.

POPULATION OF JANUARY 1ST, 1920 USED IN ALL INSTANCES.

H. W. GREEN, DIRECTOR,
BUREAU STATISTICS AND RESEARCH,
CLEVELAND HEALTH COUNCIL.
1920.

RANK - BY DEATH-RATES				CITY	POPULATION				NUMBER OF DEATHS FROM TUBERCULOSIS				DEATH-RATE PER 100,000 POPULATION AS OF JAN. 1, 1920			
WHITE		COLORED			WHITE		COLORED		WHITE		COLORED		WHITE		COLORED	
TOTAL	20-29	20-29	TOTAL		TOTAL	20-29	20-29	TOTAL	TOTAL	20-29	20-29	TOTAL	TOTAL	20-29	20-29	TOTAL
1	1	2	4	LOS ANGELES, CALIF.	546,864	97,791	6,053	29,809	965	218	40	120	176	223	661	403
2	5	10	11	NEW ORLEANS, LA.	285,916	57,800	22,691	101,303	452	89	113	361	158	154	498	356
3	3	1	1	CINCINNATI, OHIO.	371,097	68,024	6,898	30,150	499	120	52	152	134	176	754	504
4	4	3	5	NASHVILLE, TENN.	82,703	16,237	7,433	35,639	109	28	48	139	132	173	646	390
5	10	16	6	NEW YORK, N. Y.	5,469,463	1,077,100	44,476	160,585	6,629	1,539	179	608	121	143	402	379
6	6	13	7	PHILADELPHIA, PA.	1,688,180	317,220	34,046	135,599	2,018	478	159	506	120	151	467	373
7	2	22	20	LOUISVILLE, KY.	194,769	37,247	8,867	40,122	226	70	31	107	116	188	358	267
8	14	17	21	RICHMOND, VA.	117,574	24,256	12,742	54,093	136	29	50	135	116	120	393	250
9	7	9	10	BALTIMORE, MD.	625,130	119,068	25,829	108,696	714	178	131	391	114	149	507	360
10	12	8	8	MEMPHIS, TENN.	101,113	21,148	15,219	61,288	111	26	81	227	110	125	532	371
11	9	18	16	PITTSBURG, PA.	550,261	102,660	8,763	38,082	595	147	34	114	108	143	388	299
12	8	7	9	INDIANAPOLIS, IND.	279,411	54,685	7,650	34,783	288	81	42	127	103	148	550	365
13	11	5	2	CLEVELAND, OHIO.	762,026	153,341	10,175	34,815	734	197	59	149	96	128	580	428
14	20	12	15	NORFOLK, VA.	72,226	16,657	11,331	43,551	69	13	53	133	96	78	468	305
15	15	19	17	DETROIT, MICH.	952,065	226,659	14,227	41,613	892	269	54	124	94	119	380	298
16	13	14	13	CHICAGO, ILL.	2,569,169	509,035	29,158	112,536	2,287	625	126	371	88	123	432	330
17	19	20	19	WASHINGTON, D. C.	326,880	76,197	24,855	110,711	282	62	93	309	86	81	374	279
18	17	23	23	ATLANTA, GA.	137,786	30,740	16,400	62,831	118	29	43	102	86	94	262	162
19	21	21	22	HOUSTON, TEX.	104,268	22,438	8,983	34,008	86	16	33	69	82	71	368	203
20	18	6	5	KANSAS CITY, MO.	293,517	60,797	7,444	30,895	238	51	41	131	81	84	551	424
21	16	15	18	ST. LOUIS, MO.	702,615	138,408	16,026	70,282	560	142	65	207	80	103	406	295
22	22	4	12	DALLAS, TEX.	134,988	32,364	6,320	24,088	97	18	39	83	72	56	618	345
23	23	11	14	BIRMINGHAM, ALA.	108,550	22,987	17,622	70,256	73	12	87	219	67	52	494	312

The ratio between the 20–29 year specific tuberculosis death-rate for the white and colored for other cities shows considerable variation as indicated below:

Number of Times Larger the Colored Is than the White for the 20–29 Year Age-Specific Death-Rate

Los Angeles, Calif.	3	Cleveland, O.	4
New Orleans, La.	3	Norfolk, Va.	6
Cincinnati, O.	4	Detroit, Mich.	3
Nashville, Tenn.	4	Chicago, Ill.	4
New York, N. Y.	3	Washington, D. C.	5
Philadelphia, Pa.	3	Atlanta, Ga.	3
Louisville, Ky.	2	Houston, Tex.	5
Richmond, Va.	3	Kansas City, Mo.	7
Baltimore, Md.	3	St. Louis, Mo.	4
Memphis, Tenn.	4	Dallas, Tex.	11
Pittsburgh, Pa.	3	Birmingham, Ala.	10
Indianapolis, Ind.	4		

There seems to be no correlation between the white and colored specific death-rate for the 20–29 year age-group, as is indicated by the fact that the coefficient of correlation is only 0.34 ± 0.12 with a large relative probable error.

In the case of the 20–29 year age-specific death-rate in the cities shown in the table the average for the white is 125, with a range of 171 and a standard deviation or measure of the scattering tendency of 43, which is 34 per cent of the mean, while for the colored the average is 482, with a range of 359 and a standard deviation of 115, which is 24 per cent of the mean. It is thus seen that a considerable variation exists between the rates in the various cities, which indicates that the economic conditions, housing, environment and other variable factors exert a large influence upon tuberculosis mortality.

STUDIES ON VITAL STAINING IN EXPERIMENTAL TUBERCULOSIS[1]

RICHARD H. JAFFÉ

The staining of living tissues with dyes, which, due to their chemical constitution and physical properties, enter the cells without causing severe damage, has become a method widely used in modern experimental morphology. Not only does this method bring out structures otherwise less distinct, or invisible, and reveal relations between groups of cells formerly unknown, but it also yields information about intricate intracellular processes.

Vital staining and permeability constitute two problems closely related to each other. The entering of an acid stain into a cell depends upon whether the cellular surface is permeable to it or not. The cell will not stain if its surface is impermeable. In the highly permeable intestine of suckling mice, the epithelial cells store India ink that has been fed. This phenomenon disappears when the nutrition is changed. No storage of ink in the intestinal epithelium is observed after the suckling period (v. Moellendorff). Increased functional activity is connected with increased permeability. Thus, the clasmatocytes of immunized animals stain more intensively than do those of the controls (B. Portis, Schittenhelm and Erhardt, Siegmund). The exhausted, degenerated cell that has lost the physiological regulation of its permeability does not segregate the dye as granules but stains diffusely. In the dead cell the stain appears in the nucleus, which is not accessible to vital stains as long as the cell is intact.

By injecting indicators as vital stains the reactions in the cells can be determined (Peyton Rous, Petrow, Schmidtmann). Karcag, Paunz and Nemeth, using the so called electropic stains, fuchsin S, water-blue and light green, tried to detect the electrostatic charge of the cell colloids.

Studying a problem with the aid of vital staining two facts should be kept in mind. First, the administration of the dyes, though ap-

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parently harmless, changes the conditions in the animal that has been stained; cells become mobilized, and the storage of the dye may affect their functions. Second, cells whose vitality, or whose relation to their surroundings have been changed, may respond to the vital stain differently than do normal cells; a cell, normally refractory to the dyes, may stain when under abnormal conditions. The contrary should also be considered.

In this paper an attempt will be made to show the value of vital staining for the study of tuberculosis. The histiogenesis of the cells of which tubercles are composed will not be described. That has often been done, and reference is made to the publications of Goldmann, Kiyono, Joest and Emshoff, Oppenheimer, Evans, Bowman and Winternitz, Rondoni, Sewell, Foot, and M. R. Lewis, Willis and W. H. Lewis. Neither will the therapeutic effect of the substances injected be discussed, since special studies have been devoted to this problem (Lewis, DeWitt, Corper).

The experiments described were intended to answer the following questions: In a vitally stained animal the cells that are involved in building up the tubercles differ from the normal as their cytoplasm is filled with dye granules. Does this influence the formation of the tubercles? Further, the cells of a tubercle are under abnormal conditions. Does this affect their affinity to the stains?

METHODS

Adult rabbits of about 3,500 gm. in weight were used. Each series consisted of normal and splenectomized animals. The stains injected were India ink and saccharated iron oxide. India ink is a suspension and not a colloidal solution, but it has the advantage of accumulating in the body most intensively because hardly any is excreted. Two to 4 cc. of ink diluted with an equal amount of sterile saline solution were given intravenously every second day.

Iron was chosen because of its being a substance which can be called familiar to the body cells. The reticulo-endothelial cells, in particular, are intimately associated with the internal iron metabolism.

Two to 3 cc. of a 20 per cent solution were injected intravenously. Saccharated iron oxide as vital stain was first used by Eppinger, and was later studied by H. Pfeiffer and Boerner-Patzelt. It is a highly colloidal ferri-hydroxide, and the saccharose acts as protecting colloid.

It contains 2.8 per cent iron (Morawitz), and dissolves easily in distilled water. If the solutions are carefully and uniformly prepared, they are not toxic (H. Pfeiffer), and large doses can be given over a long period of time. The saccharated iron behaves in the body like an acid vital stain. The iron deposited in the cells stands out very distinctly as blue granules when the sections are treated after the Prussian-blue or Turnbull-blue methods.

The strain of tubercle bacilli used was bovine and of medium virulence. Twenty milligrams were injected into the ear vein.

THE RELATION BETWEEN VITAL STAINING AND THE DEVELOPMENT OF TUBERCLES

Since Lepehne and Eppinger published their first observations on a functional elimination of the reticulo-endothelial cells, the so called "blockade," by storing colloidal silver or iron, a large number of studies have been made to find out whether such a functional elimination is possible or not. The extensive literature has often been reviewed lately. Suffice it to say here that an agreement has not yet been reached. In various experiments the influence of vital staining upon the different assumed functions of the histiocytes was investigated. Lepehne, Eppinger, Elek, Kodama and others, made the change in the formation of bile pigment the subject of their studies. The papers of Bielig and Isaac, Rosenthal, Standenath, Gay and Clark, Neufeld and Meyer, Fraenkel and Grunenberg deal with the production of the specific antibodies; that of Moldowan and Zolog and of Schittenhelm and Erhardt with the anaphylactic shock. Seifert, Oeller, Katsuma, Saxl and Donath, and others, determined the effect upon phagocytosis.

In the earlier experiments on vital staining and tuberculosis the dye was usually injected together with the bacilli, or after the infection. Under these conditions the possibility of a functional elimination of the stem-cells of the tubercle was not considered. Most of the authors noted no difference between the stained and the control animals. Seifert, Foot, Oeller and Haythorn described phagocytosis of tubercle bacilli by cells containing dye granules. Only Beitzke mentioned the relatively light course of the tuberculosis in guinea pigs vitally stained, and Corper, Starry and Lurie mentioned that intravenous injections of India ink seemed to have favorably influenced a mild and protracted infection in rabbits.

In our series the rabbits were first injected for from 3 to 4 weeks with India ink or saccharated iron. After this time the coal or iron deposits in the histiocytes were most intense, as revealed by control animals that had been killed for the purpose of obtaining information about the extent of the vital staining.

In the control rabbit which had received the iron the Kupffer cells of the liver were enlarged and crowded with iron granules. They were always attached to the capillary wall, and showed no tendency to become free. In the spleen, large iron-filled cells were present in the lumina of the sinuses, while the lining endothelium contained only a faint trace of the metal. Many coarse iron granules were seen in the reticular cells of the pulp, and finer ones in those of the Malpighian bodies. The bone-marrow showed a large number of granules in the endothelial and reticular cells. In the lung relatively few iron cells were found. They were located in the alveolar septa between the walls of the alveoli and the capillaries. Iron was never observed in the capillary endothelium, nor in cells lying free in the lumina of blood vessels.

In the lymph nodes, especially in those about the hilum of the liver, the endothelial cells of the medullary sinuses and some of the reticular cells contained iron.

The distribution of India ink in the body has often been described (Boerner-Patzelt, Corper and associates, Foot, F. Herzog, Kiyono, McJunkin, Nagao, Perman, Sewell, Simpson, Westhues, and others). Special attention has been devoted to the localization of the coal granules in the lung. The origin of the pigmented cells of the lung from the alveolar epithelium (Sewell, Westhues) has been denied, and the two possibilities mainly discussed are that the coal is either deposited in the endothelial cells of the capillaries (Perman, Foot); or that the ink cells originate from the monocytes of the blood (M. R. Lewis), or from the reticuloendothelium of the liver, spleen, etc., and are carried into the lung by the blood-stream (Kiyono, Boerner-Patzelt, Nissen, Simpson). This contradiction of opinion is readily explained by the fact that the histological changes in the lungs of animals injected with India ink are very complicated. The pigmentation of this organ is different from that of the liver, spleen, bone-marrow, etc., in that the ink injected into the ear-veins partly precipitates in the smallest vessels of the lung, where it forms small thrombi, most pronounced in the basal parts of the lower lobes. These ink thrombi have been observed by several inves-

tigators (Perman, Boerner-Patzelt, Kiyono, Nissen, Westhues), and have recently been described by M. R. Lewis in the frog's lung.

In a separate group of experiments the fate of the ink thrombi was followed. Very thin paraffin sections (not over 3μ) of lung tissue fixed with 85 per cent alcohol, and stained with Pappenheim's methyl-green-pyronine solution, are found to be most suitable. These sections show beyond doubt that the endothelial cells of the alveolar capillaries are free from coal. They are also free when they border on an ink thrombus which fills the capillary lumen. However, branched wandering cells which surround the capillary become mobilized. They migrate into the vessel, break up the thrombus, and phagocyte the debris. The cells laden with coal either return to the interstitial tissue, or they are carried away by the blood-stream. On the other hand, a large number of ink cells from other sources are brought into the lung with the blood. These cells often stick to the capillary wall; they sometimes pass through the capillary wall, but they always remain discernible from the capillary endothelium.[2]

The findings in the other organs may be summarized briefly. The Kupffer cells of the liver are filled with black granules; the latter become rounded, and are often seen free in the lumen. They tend to form compact nodules consisting of from 12 to 20 cells, which appear as black patches. Many black cells are seen in the larger vessels and in the periportal connective tissue, which is increased.

The splenic pulp is full of cells with large masses of black granules. These cells are often arranged in groups, and similar findings are obtained in the Malpighian bodies. The endothelial cells of the sinuses are enlarged, and contain fine coal dust. Only a few ink cells are seen in the lumina of the sinuses. The bone-marrow is black from the coal deposits in the reticular and endothelial cells. Much pigment is present in the histiocytes of the abdominal lymph nodes. Less coal is found in the thoracic lymph nodes. In the kidneys the tufts of the glomeruli and the capillaries about the *tubuli* contain ink cells.

After the vital staining had reached the intensity just described the animals were infected with tubercle bacilli. The injections of the ink and iron were continued, and the animals were killed by ether after 20, 21, 28, 33, 41, 52 and 66 days.

The macroscopical findings were those typical of tuberculosis of the

[2] The increase of the pigmentation of the lung after splenectomy, described by Foot, was not evident in our experiments.

rabbit, and the extent of the lesions and the number of the organs involved varied only according to the interval between infection and death. In the splenectomized rabbits the tubercles in the lungs were relatively small and scanty. But the course of the disease did not seem to have been markedly changed by the removal of the spleen (see also Foot).

From the histological examination of the different organs the fact was evident that the stem-cells of the epithelioid cells, though crowded with iron or ink, had not lost the ability to react against the tuberculous infection. There was no difference between the stained and control animals as to the cellular composition of the specific lesions, or as to their size and regressive changes. The iron-carrying perivascular cells of the lung gave rise to the formation of epithelioid cells, as they did when unstained. The Kupffer cells of the liver, the reticular cells of the spleen and lymph nodes, filled with iron, grew, proliferated, and arranged themselves to form typical tubercles. No inhibitory influence could be detected.

The changes in the nodular accumulations of ink cells in the spleen, lymph nodes, lung, and especially in the liver, were most striking. These nodules gradually became transformed into tubercles and their histological picture changed, the ink granules becoming smaller and more loose.

With the increase in size of the tubercles, the amount of the stored material diminished. The iron, in particular, was almost completely absent when central necrosis started.

THE VITAL STAINING OF THE TUBERCLE

The statement has been made that the stain gradually disappears when the tubercles grow larger. The iron granules are distinct only in the small tubercles of certain organs, while in the larger lesions an iron cell is only occasionally found. The coal pigmentation persists much longer, but in the tubercles with caseation the single epithelioid cells show only a few fine black granules, and there are always some cells free from them.

Similar observations were made by other investigators, and were usually explained on the basis of a diminished vitality of the cells, causing loss of the function to store stains or coal-dust as granules. In his experiments on avian tuberculosis Goldmann found that the pyrrol cells exposed to tubercle bacilli gave off the dye. Kiyono saw the reason for the absence of carmine in the larger tubercles of rabbits stained with

this dye in a functional impairment of the histiocytes, which had transformed themselves into epithelioid cells. Soper said that the Kupffer cells, immediately on exposure to the tubercle toxin, lose in part their capacity to take the vital dye in their protoplasm. The absence of vital stains in fully developed tubercles has also been noted by Schlecht (lithioncarmine), Rondoni (trypan-blue), Westhues (lithioncarmine), Corper and associates (India ink).

This explanation, however, is not satisfactory, because the cells that have taken in a vital stain do not lose it when undergoing degeneration. The granules clump together, their arrangement becomes irregular, and their number may be diminished. But the cells remain stained, and the granular deposits are gradually replaced by a diffuse infiltration of the cell body. These changes are very distinct in the giant cells, which, no doubt, are cells of diminished vitality. In the giant cells the iron granules accumulate in the periphery, inside of the wreath of the nuclei. Besides, the whole cell appears deep blue when treated with potassium ferrocyanide and hydrochloric acid.

The India ink is also retained by the degenerated cells. Here, of course, a diffuse staining does not occur. The observation has even been made that cells, which normally do not store the coal, may do so when injured. Thus, black granules may be found in the foamy liver cells of rabbits that have received very many injections of the ink, or have suffered from a more advanced tuberculosis or from bronchopneumonia. In degenerated cortical cells of the adrenals, too, ink granules are visible (see also Nagao).

Comparing the staining of the tubercles in the different organs, one finds that its intensity depends upon the number of the preëxistent stained cells and upon the localization of the tubercles. There are but a few iron cells in the lung, and the smallest lesions in this organ are already practically free from the metal. In the liver much iron is seen in the intracapillary tubercles, while the tubercles that originate in the septa are only poorly stained. The small tubercles of the thoracic lymph nodes contain a few fine iron granules, while those in the abdominal lymph nodes are full of them, especially when they are located near a sinus. In the spleen the tubercles in the pulp show much more iron than those in the Malpighian bodies.

In the lungs of the rabbits injected with India ink the intensity of the pigmentation is most pronounced in the tubercles that have developed near ink thrombi that are being removed by macrophages. In other places the epithelioid cells contain much less coal.

The disappearance of the dye from the growing tubercles cannot therefore be due to a lessened vitality of the cells. In this connection attention should be called to the fact that the vital staining of the cells is determined by the concentration of the dye in the surrounding fluids. After intravenous injection the cells receive the stain mainly from the blood. The acid vital stains, and similar to them the highly colloidal iron, circulate in the blood for only a short time, because they are soon excreted through the kidneys, liver, and, so far as the iron is concerned, especially through the intestine. The coal particles of the ink are taken up by the phagocytic cells of the liver, spleen, etc., within the first hours after the injection. An intimate contact between the cells and the fluid containing the dye is therefore essential to secure the vital staining.

The tubercles are avascular. The proliferating cells lose the connection with the blood on account of their arrangement in nodular formation, into which the vessels do not extend. The stain passes the cells in quantities too small, in concentrations too low, to be taken up by them, though the necrotic centres may retain it, as sections are stained in highly diluted solution of a dye.

The dye that is found in the epithelioid cells is not actively taken in. The stem-cells transmit to the next generations the dye they have stored. With the progress of the new formation of epithelioid cells, the granules are distributed over an increasing number of cells, and the amount of dye in the single cells is necessarily diminished. That is the reason why, as stated above, the tubercles appear better stained in the organs that display a very intensive storage of the stain.

DISCUSSION

The origin of the tuberculous epithelioid cells is still a matter of discussion. Recent investigations point toward the blood monocytes, and this opinion is also found in the older literature. This theory, however, is not in accord with the facts brought forward by a number of investigators in favor of the local histiocytes. But there is no doubt as to the ability of the stem-cells to phagocyte small corpuscles and to store certain colloidal substances as granules. At the height of these functions the stem-cell retains its potency to react specifically against the tuberculous infection. A functional elimination does not exist, so far as the formation of the tubercles is concerned.

Vital staining throws light upon the conditions in the tubercles. Though the dye penetrates to the centre and stains the necrotic masses,

as described by Kiyono, Lydia DeWitt, Rondoni, and others, it is not taken up by the living tuberculous tissue. There is, however, a difference between vital stains and colloidal iron, in that the latter is not demonstrable by means of microchemical reactions in the area of necrosis.

The absence of the dye from the epithelioid cells does not result from a functional impairment. In the literature on the histiocytes it has been reported that certain alterations render the cells refractory to the vital stain (Paschkis, Hess and Goldstein). These observations, however, need further confirmation, since irritated cells either show increased quantities of the dye, or an irregular arrangement of the granules or a diffuse staining.

Comparative studies of the staining of tubercles in the different stages and in the different organs rather indicate that it is a deficiency in the circulation that makes it impossible for the dye to pass the cells in a quantity necessary for intracellular granular segregation. The small quantities of stain found in the tubercles are derived from the granules stored by the stem-cells.

In a preceding paper the observation has been reported that cholesterol, which, after repeated oral administration, accumulates in high concentration in the blood and so secures better conditions for storage in the tubercles, also appears in large quantities in the epithelioid cells of the lesions with central necrosis.

These facts may help to explain the failure of an effective chemotherapy of tuberculosis with dye compounds (DeWitt, Lewis). The dye is present in the necrotic centres. Dye-carrying cells may accumulate about the tubercle, but the zone of the active defense processes remains free from it. Bacilli engulfed by cells are not exposed to the compound because the latter does not enter the cells.

CONCLUSIONS

Most intensive vital staining of rabbits with India ink or colloidal iron does not prevent the formation of tubercles. This observation is not in accordance with the possibility of a functional elimination of cells by the granular storage of foreign material.

The decrease of the dye in the growing tubercle, and its absence under certain conditions in larger tubercles, does not result from a diminished vitality of the epithelioid cells, but is due to the lack of proper circulation.

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THE REACTION AND GROWTH CURVES OF CULTURES OF TUBERCLE BACILLI[1]

R. R. HENLEY[2]

During the course of a protracted series of chemical studies of cultures of *B. tuberculosis*, it was noted that the reaction curves varied considerably, even though the cultures were of the same strain of bacilli and grown on the same culture medium and under conditions which apparently were identical. Others have noticed similar variations, and different explanations have been offered, none of which seem adequately to explain the apparent inconsistencies in all cases. The work described in this paper was undertaken with the object of ascertaining, if possible, the cause of these variations. No attempt is made to compare the reaction curves of the human type with those of the bovine type, nor is the question of the mechanism of acid production included within the scope of the experiments.

In these experiments there has been some variation in the composition of the culture medium, but for the most part attention has been directed to the influence exerted by the surface area of the cultures and the weight of bacteria produced per flask. In a number of experiments in which the reaction of the cultures was determined at the beginning and at the end of the period of growth, which varied from 6 to 10 weeks, it was found that there appeared to be no regular relationship between the total weight of bacteria produced and the reaction of the cultures at the end of the growth period. Accordingly, the series of experiments described in this paper was carried out. The surface area of the cultures and the composition of the broth were varied, and the weight of bacteria produced and the hydrogen-ion concentration were determined weekly.

EXPERIMENTAL

The broth used in these experiments was made by infusing one part of ground lean beef in two parts of water for three hours at 50°C. The

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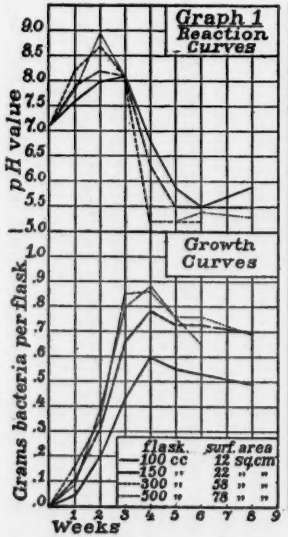
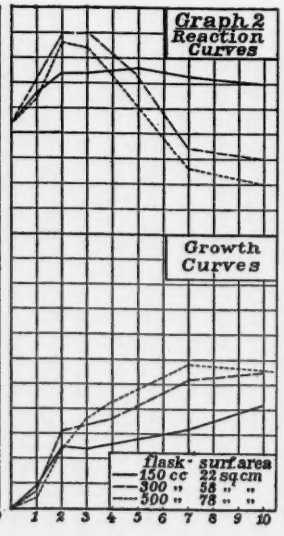
infusion was heated to boiling and filtered, and 1.0 per cent peptone and 0.5 per cent potassium biphosphate were added. In some cases 0.5 per cent sodium chloride was added in place of the phosphate, and in one experiment iron salts were added. The reaction was adjusted to +0.6 (100 cc. of broth required 0.6 cc. N/1 NaOH for neutralization). Seven per cent of glycerine was added, the broth was boiled for one hour and filtered, and 100 cc. portions were placed in Erlenmeyer flasks and autoclaved at 12 pounds pressure for 20 minutes. The pH value of media prepared in this manner was approximately 7.3.

Experiment 1: In order to obtain cultures of different surface area, Erlenmeyer flasks of four different sizes, namely, 100, 150, 300, and 500 cc., were used in this experiment. Eighty cubic centimetres of a sodium-chloride broth were placed in each of the 100-cc. flasks, and 100 cc. in each of the other flasks. The average surface areas of the cultures of the several groups are shown in graph 1. Each flask was inoculated with *B. tuberculosis*, human type (120). At the end of the first week of incubation and at weekly intervals thereafter, one flask of each group was withdrawn, sterilized and filtered through tared hardened papers. The pH value of the filtrate was determined colorimetrically. Each filter was washed with from 300 to 500 cc. of distilled water. After washing, the filters with the contained bacteria were transferred to tared aluminum dishes provided with covers and dried at 100°C. until the loss in weight after 2 hours did not exceed 2 mgm. The weight of bacteria was then calculated. The results are presented in graph 1.

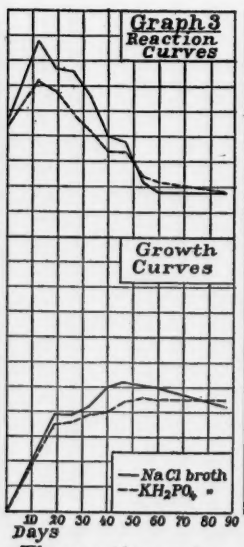
Smith has shown that there are two stages in the reaction curves of cultures of *B. tuberculosis:* a first stage, during which the cultures increase in alkalinity, and a second stage, in which the reaction remains unchanged or becomes more acid. The two stages may be seen in the reaction curves of graph 1. This graph also shows that growth took place in two stages: first, a stage in which the rate (the amount produced in a given time) was relatively high, and second, a stage during which growth either ceased or became greatly reduced in rate. The two stages of the growth curves are reflected by the reaction curves; in general, the period during which the reaction curves rise is also the period of most rapid growth and in point of time the second, or acid stage of the reaction curves, corresponds with the second growth stage, that is, the decrease in pH value began about the time when the end of the first growth stage was reached. In graph 1 the reaction curves of



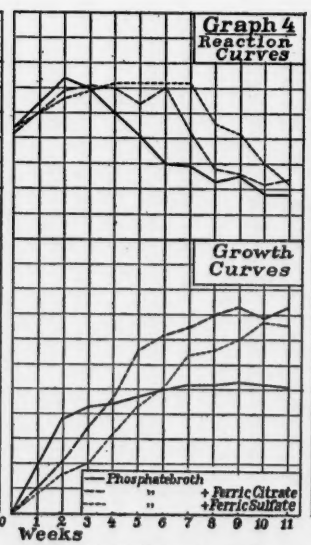
The effect of surface area on the reaction and growth curves of sodium-chloride cultures of B. tuberculosis, human type



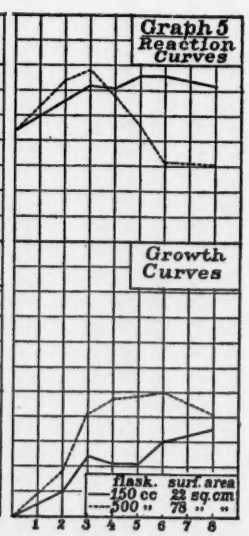
The effect of surface area on the reaction and growth curves of phosphate cultures of B. tuberculosis, human type



The reaction and growth curves of cultures of the same surface area of B. tubercul., human type, on phosphate and sodium-chloride broth



The effect of ferric salts on the reaction and growth curves of phosphate cultures of B. tuberculosis, human type



The effect of surface area on the reaction and growth curves of phosphate cultures of B. tuberculosis, bovine type

the cultures in the 300-cc. and 500-cc. flasks almost coincide, and this likewise holds true for the growth curves. The cultures in these flasks made the greatest growth, and the greatest increase in pH value during the first three or four weeks. They were the first to become acid, and the first to reach a pH value below 6.0.

Experiment 2: This experiment was similar to experiment 1, differing only in the use of KH_2PO_4 instead of NaCl in the broth, and in the use of only three groups of flasks, namely, of 150-cc., 300-cc. and 500-cc. capacity. Results are shown in graph 2.

The reaction and growth curves shown in graph 2 are less abrupt than those in graph 1, but they nevertheless show corresponding stages and similar relations. The pH value of the culture in the smallest flask, after reaching 8.2 at the end of the second week, never fell below 8.0. The graph shows that the weight of bacteria in these smallest flasks during the first growth stage did not exceed 0.25 gm. per flask. This suggests that in order to produce a sharp drop in pH value during the second reaction stage, it may be necessary for the growth to reach a certain maximum during the first growth stage.

Experiment 3: Two different broths were used, but the flasks were all of the same size (300 cc.). The broths differed only in that one contained added potassium biphosphate and the other added sodium chloride. Each flask contained 100 cc. of broth, and all were inoculated with *B. tuberculosis*, human type (120). Determinations of pH value and weight were made on two or three flasks at the intervals indicated in graph 3. In duplicate determination the weights of bacteria per flask usually did not vary more than 0.05 gm., nor the pH values more than 0.2. The results appear in graph 3.

In graph 3 the growth curves are very similar, and the reaction curves are almost parallel. It is interesting to note that the culture on the sodium-chloride broth reached a higher pH at the end of the 12 days and that at the same time the growth in these cultures was slightly greater. However, the differences in the reaction curves at that time may in part be accounted for by the buffer action of the phosphates. The differences between the growth and reaction curves of graph 1 on the one hand, and of graph 2 on the other, might have been attributed to the use of sodium chloride in one case and acid potassium phosphate in the other were it not for graph 3, which indicates that the controlling

factor was the rate of growth. When that is similar, as in experiment 3, the reaction curves are also similar.

The reaction and growth curves of cultures of the human type on media containing iron, which is known to influence favorably the growth of the bacilli, were studied in experiment 4.

Experiment 4: One hundred-cubic centimetre portions of a phosphate broth in 300-cc. flasks were divided into three groups. One group served as controls, ferric citrate was added to another and ferric sulphate to the third group. The amount of each salt was equivalent to 5.5 mgm. of Fe per 100 cc. of broth. Each flask was inoculated with *B. tuberculosis*, human type (D.T.). Two flasks of each lot were withdrawn from the incubator at weekly intervals and the usual determinations made. The results are shown in graph 4.

The previously observed relation between the growth and reaction curves also holds in this experiment; that is, the relative order in which the weight of growth of the different cultures increased during the first three weeks was the order in which the pH values increased during that time, and also the order in which the cultures subsequently became acid. The cultures which contained iron exhibited the two stages of the growth curve but the initial stage was less abrupt and continued for a much longer time than in cultures on plain phosphate and sodium-chloride broths. The prolongation of the first stage of growth was accompanied by a delay in the downward tendency of the reaction curves.

The reaction and growth curves of cultures of *B. tuberculosis* of the bovine type were determined in experiment 5.

Experiment 5: One hundred-cubic centimetre portions of a phosphate broth (pH 7.2) were placed in each of 12 flasks of 500-cc. capacity and in each of 12 flasks of 150-cc. capacity. Each flask was inoculated with a bovine-type culture of *B. tuberculosis* and incubated. The pH values and weights of the pellicles were determined in duplicate, beginning at the second week. Results appear in graph 5.

The final pH value of the cultures of the bovine type of bacilli did not fall as low as usual with cultures of the human type; nevertheless, the relations between the reaction and growth curves were essentially the same as those observed in cultures of the human type. The cultures in the smaller flasks never became acid. It is interesting to compare the growth and reaction curves of these cultures of the bovine type

with those of the human type in the smallest flasks shown in graph 2. In the bovine cultures in the smaller flasks the total growth during the initial stage amounted to only 0.24 gm. and the reaction curve, although reaching pH 8.3, never fell below 8.0. The similarity between the reaction and growth curves of the two different types of cultures is quite striking.

In the foregoing experiments the growth of cultures of both the human and bovine types of *B. tuberculosis* proceeded in two stages, and in a

TABLE 1

The relation between the amount of growth at three weeks and the reaction at eight weeks

WEIGHT OF BACTERIA AT 3 WEEKS*	pH VALUE OF CULTURE AT 8 WEEKS	MAXIMUM WEIGHT OBSERVED	BROTH		CULTURE	GRAPH
			Surface area	Kind		
grams		*grams*	*sq. cm.*			
0.16	8.2	1.04	58	$Fe_2(SO_4)_3$	Human 120	Unreported
0.20	7.3	0.77	58	$Fe_2(SO_4)_3$	Human DT	4
0.24	8.1	0.42	22	KH_2PO_4	Human 120	2
0.24	8.1	0.35	22	KH_2PO_4	Bovine III	5
0.34	6.6	0.55	58	KH_2PO_4	Human 120	2
0.35	6.4	0.83	58	$Fe_2(C_6H_5O_7)_2$	Human DT	4
0.36	6.2	0.46	58	KH_2PO_4	Human DT	3
0.36	6.2	0.58	78	KH_2PO_4	Human 120	2
0.39	6.1	0.52	58	NaCl	Human DT	3
0.39	6.0	0.59	58	KH_2PO_4	Human 120	Unreported
0.41	6.5	0.50	78	KH_2PO_4	Bovine III	5
0.43	6.1	0.53	58	KH_2PO_4	Human DT	4
0.67	5.5	0.78	22	NaCl	Human 120	1
0.80	5.3	0.88	58	NaCl	Human 120	1
0.85	5.2†	0.86	78	NaCl	Human 120	1

* In some cases the figures for weights and pH values are averages of several flasks, in others they are taken from a single observation.

† At the sixth week.

general way these two stages of growth coincided with the two stages of the reaction curve. The more abruptly and higher the growth curves rose during the early weeks of incubation, the earlier and more sharply the reaction curves fell after reaching the maximum. Thus the weights of two cultures which had grown at different rates were in some instances approximately the same at a given time, while the reaction was quite different (graphs 4 and 5). In such cases the lower pH value was found in the culture in which the greater growth was made during the earlier

weeks of incubation. It may be of interest to note that in these, as well as in unrecorded experiments, the reaction of cultures exhibiting heavy growth approaches pH 5.0, but in the experience of the writer it has not fallen below that point.

Comparisons in the preceding experiments have been made in some cases between cultures on media of different composition and in others between cultures of different surface area. It has been suggested that there are two distinct growth stages just as there are two distinct reaction stages during the cultivation of *B. tuberculosis* on broth, and it has also been suggested that the reaction during the second reaction stage is dependent upon the rate of growth during the first growth stage. In order to compare further the effect of rate of growth during the initial stage upon the reaction during the later stage, table 1 was prepared from data used in constructing the graphs. While perhaps not essential, there are included in the table the maximum weights observed, the surface areas of the cultures and a note on the kind of broth and type of bacilli used. In this table only results from cultures of equal volume (100 cc.) are included.

This table shows that the weight of growth at three weeks varied from 0.16 to 0.85 gm., while the reaction at eight weeks varied from pH 8.2 to pH 5.2. In a general way it may be seen that the acid produced at eight weeks was dependent upon the amount of growth at three weeks. This held true regardless of surface area, of the composition of the broth and of the type of culture used.

The writer wishes to acknowledge his indebtedness to Dr. M. Dorset upon whose suggestion and under whose supervision the work was done, to Dr. F. W. Tilley for the determinations of pH values and also for his advice and suggestions, and to Dr. Angus D. MacDonald for his care in inoculating and incubating the cultures.

UNSUCCESSFUL ATTEMPTS TO CURE OR PREVENT TUBERCULOSIS IN GUINEA PIGS WITH DREYER'S DEFATTED ANTIGEN[1]

MARY NEVIN, FLORENCE R. BITTMAN AND ELIZABETH LEE HAZEN

Numerous promising preventive and therapeutic agents against tuberculosis have been and continue to be described. Upon investigation by other workers all of the claims of either the prophylactic or therapeutic merit of vaccines, serums, etc., have proved to be of dubious, if any, value. In most instances the effect of the treatment has been decidedly harmful.

When Dreyer's work on the therapeutic value of a "defatted" vaccine, made from the *Bacillus tuberculosis*, was published, it created quite an interest in the medical world, which looked forward to a more extensive repetition of the work by numerous investigators and a possible verification of all that Dreyer had claimed.

On account of the fact that Dreyer had used so few animals, and, according to our opinion, insufficient controls, we decided to repeat his experiment, using a greater number of animals and more controls, and at the same time to test the prophylactic as well as the therapeutic value of the vaccine.

In the preparation of the vaccine, Dreyer's technique was accurately followed. In some instances it was necessary to subject the bacillary bodies to three or even four defatting processes in order to render them non-acid fast and gram-negative. In no instance was the suspension used until these two criteria were fulfilled.

Vaccines: Defatted vaccines prepared by Dreyer's method:

Vaccine I: *B. tuberculosis*, human type, mixed strains, R1 and 305.

Vaccine Ia: *B. tuberculosis*, human type, strain 305.

Vaccine II: *B. tuberculosis*, bovine type, mixed strains, N. Y. S. V. C., Saranac (B) and Ravenel, Penn.

Vaccine III: *B. tuberculosis*, bovine type, Ravenel strain.

[1] From the Research Laboratories, Department of Health, New York City, and the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Control Vaccines:

Vaccine IV: *B. tuberculosis*, human type, mixed strains R1 and 305. Put through the same process as the defatted vaccines, with the exception that physiological salt solution was used instead of the formaldehyde and acetone.

Vaccine V: *B. tuberculosis*, human type, mixed strains, R1 and 305. The microörganisms were suspended in physiological salt solution in the proportion of 0.1 gm. in 10 cc., and put in the Arnold sterilizer for 1 hour at 100°C. The suspension was then centrifugated for 5 minutes at low speed. The antigen was prepared from the supernatant fluid containing the even suspension of bacillary bodies and diluted with physiological salt solution so that, after correction for the clumps and particles of bacilli thrown down by centrifugation, 1 cc. contained 0.2 mgm. of bacilli.

The strains used in the preparation of vaccines I, Ia, III, IV, and V were obtained from our stock cultures, New York City Research Laboratories. The strains used in vaccine II were kindly sent us from the New York State Veterinary College, Cornell University, Ithaca, New York.

Guinea pigs weighing from 300 to 400 gm. were used in all of the experiments, no special attention being paid to their sex. Inoculations of cultures were made subcutaneously in the left inguinal region. Injections of vaccine were made subcutaneously over the abdomen.

Suspensions and dilutions were made so that the total amount injected was always 1 cc.

Experiment 1: Forty-nine guinea pigs were inoculated with 0.1 cc. of sputum that showed, microscopically, 1 to 3 tubercle bacilli per field. Three weeks later the animals were divided into 5 groups and treated as follows:

Group A: 10 received an injection of 0.1 mgm. of vaccine II, bovine type (mixed strains) defatted.

Group B: 10 received the same amount of vaccine I, human type (mixed strains) defatted.

Group C: 10 received the same amount of control vaccine IV, human type, mixed strains "processed."

Group D: 10 received the same amount of control vaccine V, human type, mixed strains "unprocessed."

Group E: 9 animals were left untreated to serve as controls.

Results

Palpable left inguinal lymph nodes developed in all of the animals within 15 days.

Group A: 5 died in 3 weeks, and 5 in 4 weeks after infection.

Group B: 6 died in 3 weeks, and 4 in 4 weeks after infection.

Group C: 6 died in 3 weeks, and 4 in 4 weeks after infection.

Group D: 3 died in 3 weeks, and 7 in 4 weeks after infection.

TABLE 1

Experiment 1

GROUP	NUMBER OF GUINEA PIGS	INOCULUM	AMOUNT	INTERVAL BETWEEN INOCULATION AND VACCINE TREATMENT	VACCINE	DEATHS, TIME AFTER INFECTION
			cc.	*weeks*		
A	10	Tuberculous sputum	0.1	3	II, bovine type, defatted (mixed strains)	5 in 3 weeks 5 in 4 weeks
B	10	Tuberculous sputum	0.1	3	I, human type, defatted (mixed strains)	6 in 3 weeks 4 in 4 weeks
C	10	Tuberculous sputum	0.1	3	IV, human type (mixed strains), "processed" control	6 in 3 weeks 4 in 4 weeks
D	10	Tuberculous sputum	0.1	3	V, human type (mixed strains), "unprocessed" control	3 in 3 weeks 7 in 4 weeks
E	9	Tuberculous sputum	0.1	3	Untreated	1 in 1 month 4 in 1½ months 4 in 2 months

None of the animals survived long enough for a second injection of vaccine, which was due one week after the first dose.

Group E (controls): 1 died in 1 month, 4 in 1½ months, and 4 in 2 months.

All of these untreated controls survived longer than the animals that received the vaccines. The animals receiving the defatted vaccines

died as quickly as those receiving the "processed" and "unprocessed" control vaccines.

Experiment 2: Forty-two guinea pigs were inoculated with 0.1 mgm. of *B. tuberculosis*, bovine type (Ravenel). Two weeks after inoculation the animals were divided into 5 groups and treated as follows:

Group A: 8 received 0.1 mgm. of vaccine I (defatted human type, mixed strains).

Group B: 8 received 0.1 mgm. of vaccine II (defatted bovine type, mixed strains).

Group C: 8 received 0.1 mgm. of control vaccine IV ("processed" human type, mixed strains).

Group D: 8 received 0.1 mgm. of control vaccine V ("unprocessed" human type, mixed strains).

Group E: 10 were left untreated to act as controls.

Results

Palpable left inguinal lymph nodes developed in all animals in from 7 to 10 days, and suppurative lesions were noted in 24 and 48 hours after vaccine injection, in the inguinal region in all animals of groups A, B, C and D.

Group A: 1 animal died in 1 week, and 7 in 4 weeks after infection.

Group B: 4 animals died in 3 weeks, and 4 in 4 weeks after infection.

Group C: All 8 animals died within 4 weeks following infection.

Group D: All 8 animals died within 4 weeks following infection.

Group E (controls): Enlarged inguinal lymph nodes developed within 1 week, and suppurative lesions within 3 weeks after inoculation with the culture. Two animals died in 1 month, and the remaining 8 in 2 months.

Experiment 3: Twenty-four guinea pigs were inoculated with 0.005 mgm. of *B. tuberculosis*, bovine type (Ravenel). Two weeks later the animals were divided into 2 groups and treated as follows:

Group A: 18 received 0.1 mgm. of vaccine II, bovine type (mixed strains).

Group B: 6 were left untreated for controls.

Results

Group A: Palpable inguinal lymph nodes developed in from 10 to 13 days after infection, and suppurative lesions in 1 to 4 days following

TABLE 2

Experiment 2

GROUP	NUMBER OF GUINEA PIGS	INOCULUM	AMOUNT	INTERVAL BETWEEN INOCULATION AND VACCINE TREATMENT	VACCINE	DEATHS, TIME AFTER INFECTION
			mgm.	*weeks*		
A	8	*B. tuberculosis* bovine, Ravenel	0.1	2	I, human type, defatted (mixed strains)	1 in 1 week 7 in 4 weeks
B	8	*B. tuberculosis* bovine, Ravenel	0.1	2	II, bovine type, defatted (mixed strains)	4 in 3 weeks 4 in 4 weeks
C	8	*B. tuberculosis* bovine, Ravenel	0.1	2	IV, human type (mixed strains), "processed" control	8 in 4 weeks
D	8	*B. tuberculosis* bovine, Ravenel	0.1	2	V, human type (mixed strains), "unprocessed" control	8 in 4weeks
E	10	*B. tuberculosis* bovine, Ravenel	0.1	2	Untreated	2 in 1 month 8 in 2 months

TABLE 3

Experiment 3

GROUP	NUMBER OF GUINEA PIGS	INOCULUM	AMOUNT	INTERVAL BETWEEN INOCULATION AND VACCINE TREATMENT	VACCINE	DEATHS, TIME AFTER INFECTION
			mgm.	*weeks*		
A	18	*B. tuberculosis* bovine, Ravenel	0.005	2	II, bovine type, defatted (mixed strains)	4 in 3 weeks 13 in 1 month 1 in 1½ months
B	6	*B. tuberculosis* bovine, Ravenel	0.005		Untreated	2 in 2 months 4 in 3 months

vaccination. Four died in 3 weeks, 13 in 1 month, and 1 in 1½ months after infection. One guinea pig received a second dose of the vaccine (0.2 mgm.) 2 weeks after the first dose. The suppurative lesion in the inguinal region gradually healed with a crust formation. Nineteen days after the second dose of vaccine the animal was found dead. *Necropsy:* The inguinal lymph node appeared smaller, and the number of tuberculous nodules in the spleen, liver and lungs was not so great as in the guinea pigs which received the same dose of culture and which died after the first vaccination.

Group B: The 6 untreated controls developed palpable inguinal lymph

TABLE 4

Experiment 4

GROUP	NUMBER OF GUINEA PIGS	INOCULUM	AMOUNT	INTERVALS BETWEEN INOCULATION AND VACCINE TREATMENT	VACCINE	DEATHS, TIME AFTER INFECTION
			mgm.			
A	20	*B. tuberculosis* bovine, Ravenel	0.0005	48 hours after infection. Subsequent treatments at one week intervals	III, bovine type defatted, Ravenel strain	5 in 1 week 6 in 2 weeks 4 in 3 weeks 4 in 4 weeks 1 in 4 weeks and 3 days
B	9	*B. tuberculosis* bovine, Ravenel	0.0005		Untreated	4 in 6 weeks 2 in 2 months 3 in 3 months

nodes in 10 to 13 days, and suppurative lesions within 1 month after inoculation. Two died in 2 months and 4 in 3 months after infection.

Experiment 4: Twenty-nine animals were inoculated with 0.0005 mgm. of *B. tuberculosis*, bovine type (Ravenel), and divided into 2 groups, as follows:

Group A: 48 hours after infection the animals were vaccinated with 0.2 mgm. of vaccine III, bovine type, defatted Ravenel strain. Using the same amounts, these vaccinations were continued at intervals of 1 week until all of the animals had died.

Group B: 9 were left untreated as controls.

Results

Group A: All animals developed palpable inguinal lymph nodes in 10 to 12 days. Fifteen animals survived to receive the second vaccination 1 week later; 9 survived to receive the third vaccination. Four survived and were given a fourth vaccination, while 1 guinea pig lived to receive a fifth vaccination, but died 3 days following. After the second vaccination suppurative processes were apparent in the enlarged inguinal region of all animals. After the fourth vaccination the lesions in the surviving animals were entirely covered with a thick crust-like formation, which when broken showed but a slight amount of pus underneath.

Group B (controls): All developed palpable inguinal lymph nodes in 2 weeks, and suppurative lesions in from 3 weeks to 1 month; no crust-like formation was noted. Four of the animals died in 6 weeks, 2 in 2 months and 3 in 3 months, following infection.

In the foregoing experiments the defatted vaccine prepared according to Dreyer's method had failed to show any beneficial effect in the treatment of tuberculous guinea pigs.

On account of the fact that we had deviated somewhat from Dreyer's technique in the treatment of the animals as to time and dosage, the next experiment, no. 5, following accurately his dosage and intervals of treatment, was carried on.

Experiment 5: Fifty-eight guinea pigs were inoculated with 0.0005 mgm. of *B. tuberculosis*, human type, strain 305. After the development of palpable inguinal lymph nodes in 4 weeks, they were divided into groups A and B, as follows:

Group A: 38 received injections of human type defatted vaccine Ia, as follows: first dose, 0.1 mgm.; second dose, 0.2 mgm., 3 weeks after first dose; third dose, 0.1 mgm., 3 weeks after second dose. Subsequent treatments were of 0.1 mgm. every 10 days over a period of 3 months.

Group B: 20 were left untreated as controls.

Results

Group A: 28 animals died in 3 months after culture inoculation. The remaining 10 died in 4 months after inoculation. Suppurative lesions developed in about 60 per cent of the animals, but no marked crust formation was noted.

TABLE 5
Experiment 5

GROUP	NUMBER OF GUINEA PIGS	INOCULUM	AMOUNT	INTERVALS BETWEEN INOCULATION AND VACCINE TREATMENT	VACCINE	DEATHS, TIME AFTER INFECTION
			mgm.			
A	38	*B. tuberculosis* human, 305	0.0005	1st dose: 4 weeks after infection 2nd dose: 3 weeks after 1st dose 3rd dose: 3 weeks after 2nd dose. Subsequent treatments every 10 days	Ia, human type defatted 305 strain	28 in 3 months 10 in 4 months
B	20	*B. tuberculosis*	0.0005		Untreated	7 in 5 months 7 in 6 months 3 in 7 months 3 in 10 months

TABLE 6
Experiment 6

GROUPS	NUMBER OF GUINEA PIGS	VACCINE	DOSAGE AND INTERVALS	CULTURE	AMOUNT	INTERVAL	DEATHS
			mgm.		*mgm.*		
A	11	III bovine defatted Ravenel	0.2 0.1 0.1 0.2 at weekly intervals	*B. tuberculosis* bovine type (Ravenel strain)	0.0005	3 weeks after last injection of vaccine	4 in 2 weeks (before culture inoculation). After infection: 1 in 4 weeks 6 in 5 weeks
B	6			*B. tuberculosis* bovine type (Ravenel strain)	0.0005		2 in 6 weeks 2 in 2 months 2 in 3 months

Group B (controls): 7 animals died in 5 months after culture inoculation, 7 died in 6 months, 3 in 7 months, and 3 in 10 months. No suppurative processes developed in any of the animals.

Experiment 6, Prophylactic:

Group A: 11 guinea pigs were vaccinated with 4 successive doses of vaccine III, defatted bovine type (Ravenel), at intervals of 1 week. (Doses: 0.2, 0.1, 0.1, 0.2 mgm.) Twenty-four hours after the first dose of vaccine, sterile abscesses developed at the point of inoculation, which healed with a crust in 8 to 10 days. There was some swelling which persisted for 3 weeks. Four of the animals died after the third injection of vaccine (before culture inoculation). On autopsy of these 4 guinea pigs the same picture was noted in all,—necrotic areas in the subcutaneous tissues over the abdominal wall surrounding the points of injection. All organs were apparently normal, the stomach was empty, and there was extreme emaciation. Smears of the organs and necrotic tissues showed no bacilli. Three weeks after the last injection of vaccine, the 7 remaining animals were inoculated with 0.0005 mgm. of *B. tuberculosis*, bovine type (Ravenel).

Group B (controls): At the same time 6 guinea pigs were inoculated with 0.0005 mgm. of *B. tuberculosis*, bovine type (Ravenel).

Results

Group A: 1 animal developed palpable inguinal lymph nodes in 8 days, and died 4 weeks after infection. Palpable inguinal lymph nodes were noted in the remaining 6 animals in 10 days, and suppurative lesions developed 3 weeks after infection. These guinea pigs died in 5 weeks after infection.

Group B (controls): All animals developed palpable inguinal lymph nodes in 16 to 18 days. Four showed suppurative lesions in 5 to 6 weeks; 2 died in 6 weeks, 2 in 2 months, and 2 in 3 months following infection.

Postmortem findings: An autopsy was made of each animal that died and a careful record kept of the findings. Smears were made from the affected organs and examined for the presence of *B. tuberculosis*. In all instances tubercle bacilli were demonstrated in smears from the enlarged left inguinal lymph node (point of inoculation), and according to the progress of the infection they were found in other organs of the body. There was marked individual variation in the gross autopsy

findings, but these bore no relation to the intervals of the treatments or to the various antigens used. The same variation was found more or less in each group. All animals showed a markedly enlarged, caseous left inguinal lymph node, and the lymph nodes throughout were generally involved. According to the individual variation of the animals, tuberculous lesions were noted in the spleen, kidneys, liver and lungs. Some showed considerable abdominal involvement with marked adhesions.

DISCUSSION

According to the foregoing experiments, the defatted vaccine prepared according to Dreyer's method produced no beneficial results in tuberculous guinea pigs when treated either therapeutically or prophylactically. On the contrary, the animals treated with the vaccine succumbed to the infection earlier than the controls that received no vaccine treatment. This seems to indicate that the resistance of the animals is lowered by the antigen treatments. Bronfenbrenner and Straub (2), and Kettel (3) have also noted this fact in their experiments.

The vaccine treatment was generally followed by suppurative lesions, in only a few instances healing over with the crust formation mentioned by Dreyer. In no instance, however, was this formation followed by recovery. All of our animals succumbed to the infection.

SUMMARY

In our hands the defatted antigen prepared according to Dreyer's method failed to show either therapeutic or prophylactic benefit when injected into tuberculous guinea pigs. On the contrary, animals treated with the vaccine died earlier than the untreated controls.

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STUDIES IN TUBERCULOSIS

VI. LATENT TUBERCULOSIS IN CHILDHOOD, WITH REFERENCE TO TUBERCULIN REACTIONS, CLINICAL FINDINGS AND X-RAY STUDIES

FREDERICK EBERSON[1]

There is a wide gap between a frank, active pulmonary tuberculosis in children and a border-line type of infection. The latter is as elusive and vague as the numerous classifications and divergent view-points which the problem of juvenile tuberculosis has created. A latent type of infection is serious, not only because of its potential activity, but also because the absence of striking cardinal signs and symptoms tends to minimize the importance of certain data.

Tuberculosis does not differ from syphilis, typhoid fever, malaria, trypanosome or piroplasm infections with regard to latency and a "carrier" state. There is established evidence to show that micro-organisms do not lose their virulence despite habituation to the host. In diagnosing tuberculosis in children it is essential to distinguish "healed inflammatory conditions," active hilum tuberculosis, evidence of "previous infection with tuberculosis," and latent tuberculosis. There are, indeed, much confusion in terminology and loose usage of terms, due mainly to misinterpretation of immunological and biological phenomena. An early infection with tuberculosis may become latent from its beginning, an active disease may become quiescent, yet, at all events, latent tuberculosis is tuberculosis and must be recognized and treated as such. If one is to arrive at a scientific conclusion based on clinical and laboratory findings, no single factor must be dissociated from the whole. The clinical history, physical examination, X-ray study and tuberculin tests, when positively correlated, establish diagnostic criteria. However, the absence of certain signs or symptoms need not invalidate the conclusion provided the essential points be correlated. What these essentials should be is still open to question. A study of a large series of cases over a period of years may give us a standardized plan.

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Something may be learned from a study of a selected group of cases which lend themselves to an interpretation based on positive facts in the history. This method is preferable because an array of positive findings is of more value in a study in which negative data may mean nothing. Whereas a group of children may happen to be "normal" by virtue of certain negative findings, it would seem that positive characters such as definite exposure to tuberculosis, symptoms and the like, if correlated with other findings such as tuberculin reactions and X-ray studies, are more conclusive, since they are known.

CLINICAL EXPERIMENTAL INVESTIGATION

Tuberculosis in children who are between the ages of three and twelve is usually of a distinct type. The lesions rarely involve the apices of the lung. When apical tuberculosis is present it is an adult type of infection and is found, as a rule, in children under three years of age in whom the disease is general or disseminated in character. We are concerned in this study with the type of infection known correctly as "hilum tuberculosis," in which the lesions are found first in the lymph nodes about the trachea and bronchus, and the latter's large subdivisions. Such infections may involve the lymphoid elements in relation to the branches of the bronchial tree and may subsequently produce lesions in adjacent tissue. Inasmuch as "minimal" tuberculosis, so called, occurs in adults, it would be more accurate to separate this type from that larger group of juvenile infections in which the tuberculosis is confined to the bronchial lymph nodes and to the root of the lung. Hilum tuberculosis, active or quiescent, properly speaking belongs in the more inclusive group of latent tuberculosis. The problem of latency cannot be discussed without reference to reinfection and its related problems, but this phase must be deferred for the present. That autogenous infection occurs along with exogenous infection has been shown both experimentally and clinically, and pathological studies, notably by Méry (1) and Opie (2), have thrown additional light on the question. How much resistance is developed by an infection acquired in childhood will be manifested in adults by the type of tuberculosis which may develop. In children the type and extent of a tuberculous process can be gauged only poorly by the time which has elapsed between the first infection and the later manifestations. However, if we can demonstrate a subsequent infection in patients who have had the signs of a "healed"

tuberculosis, with calcification of the lymph nodes and pulmonary foci, we are justified in the conclusion that endogenous infections do develop in children who are supposed to be "negative for tuberculosis."

Clinical Material

This report is to be considered merely preliminary to more extensive studies which are suggested by the observations and data gleaned from a group of young patients. The majority of these were under observation for a period of one to three years and a small number, approximately 25 per cent, for six months to one year. Another group, about 10 per cent, had been observed for six to eight years. An attempt has been made to apply a few basic tests and criteria to a series of cases with comparable and seemingly definite points of departure.

We are dealing here with "contacts" who give indisputable histories of exposure to tuberculosis. This series of cases, 145 in number, though small because of circumstances which prevent further additions at this writing, contains a few sentient observations which may be of value in the study of juvenile tuberculosis. The early part of this work was done at the Glen Lake Sanatorium in Minneapolis, with clinical material from the Children's Building on the service of Dr. J. H. Bendes. The writer is indebted to him for collaboration and aid, especially in connection with X-ray and some of the tuberculin studies. Subsequent investigations were made in the Out-Patient Chest Clinic at the University of California.

Under clinical considerations will be enumerated history, age-groups, symptomatology, physical examination, X-ray findings and tuberculin reactions.

History: Without exception the children who came under observation in this study had a definite history of exposure to tuberculosis. Either one or both parents or relatives with an infection had come into contact with them over a period sufficiently long to warrant the likelihood of spreading the disease. In the large majority of instances, active, open cases of pulmonary tuberculosis existed in the immediate family at the time when the children were brought to the clinics.

Analysis of the histories and positive clinical data relating to the possible sources of infection, as well as to the individual juvenile cases, did not seem to bear out the general conclusions which have been made by Drolet (37) and Wahl and Gerstenberger (36) regarding the apparent

inheritance factor in tuberculosis as gauged by tuberculin reactions or by evidence of juvenile infection. Our observation may be apparent or real and the same may be said for a purely statistical study despite its impressive number of analyzed cases.

Age-groups: Regardless of the degree of exposure to infection, the greatest number of cases, 72 per cent, fell within the age limits of one to nine years, and of these 58.6 per cent were between five and nine years of age.

AGE	NUMBER	PERCENTAGE
Under 1 year	4	2.7
1–4	43	29.6
5–9	61	42.3
10–12	26	17.9
13–16	11	7.5

Symptomatology: There is a symptom-complex in children which has been widely accepted as pathognomonic of tuberculosis. In some patients all or most of these cardinal symptoms may be present at the same time. It is the usual experience, however, to meet with a few of these only, and in a certain proportion of patients to find a single complaint coupled with a vague history. As a rule, one is hard put to it to make an unequivocal statement because of such elusive symptomatology. While there is no attempt here to establish a fixed category of symptoms the following ones have been found associated so frequently as to be of significance: Lassitude, loss of weight or failure to gain, cough or hoarseness, frequent chronic "colds," lack of appetite or capricious appetite, irritability, profuse sweating of unknown origin, and cycles of rise in temperature for which an apparent cause cannot be found. In children of early school age it is not uncommon to elicit merely a history of irritability and lack of concentration in the class-room with subnormal standards in study. The actual diagnostic importance of any one symptom or combination of symptoms might be measured in a large series of cases in which the different factors are amenable to accurate correlation studies. This is a problem which merits further investigation, since, for example, it is possible for "symptom-free" children to have roentgenological evidence of definite tuberculosis, an experience which was not rare in our study.

In the group of patients under discussion the symptom-complex had

additional corroborative sources in the clear-cut histories and certain salient physical findings which were reinforced further by X-ray and tuberculin studies.

Physical findings: In our experience the methods which are of value in the diagnosis of adult tuberculosis cannot be applied to children with a similar degree of accuracy. It has been found that cervical adenopathy, while commonly associated with juvenile tuberculosis, may not be pathognomonic in all cases. Auscultation and percussion, invariably reliable when pronounced physical signs and symptoms are present, are of questionable value in the ordinary types of infection. Unless the adenopathy behind the sternum or adjacent to the vertebral column is significant, percussion gives but little aid. Here the X-ray is distinctly valuable in revealing pathological evidences of mediastinal or intrapulmonic glands. Similarly, auscultation falls short, except in those patients in whom modified voice transmission and altered breath-sounds are occasioned by marked adenopathy. In these the changes can be localized over the infraspinous and supraspinous areas and the interscapular space. The expiratory phase is markedly prolonged and the breath-sounds are quite harsh. D'Espine's sign has not been found of diagnostic value in our examinations and has not been correlated with adenopathic changes in the chest.

Roentgen-Ray Observations in Children

Much stress has been placed on the X-ray picture in juvenile patients. The extremes have been emphasized to the exclusion of a sane middle ground. In attempting to justify the importance or worthlessness of certain findings on the film, the value of other known facts is overlooked. A decisive statement must depend upon an agreement as to what constitutes a so called "normal chest" in children. From recent studies (3) roentgenologists have come to the conclusion that variations in the normal chests of children make such exact description impossible. Some of these points will be analyzed since they have a bearing on the present investigation.

Calcification of lymph nodes in and about the lung: The significance of calcification in the hilum or intrapulmonic lymph nodes has been emphasized by the pathological studies of Ghon, Harbitz, Much, Beitzke, Most, Opie, Méry and others. Although unanimity of opinion is lacking with regard to the mechanism of primary intrapulmonic infection in

children, it seems, to be well established that calcification is doubtless pathognomonic of tuberculosis. Noteworthy is the fact, shown by Opie (4), that the lesions of tuberculosis in children may be scattered in the lung substance with no special predilection, while at times a solitary focus of infection may be present. The large majority of such foci have been found in contact with the pleural surface of the lung. Bearing on the moot question as to the direction of the infection, this observation squares with the anatomic relationship of subpleural lymphatics to drainage.

Calcification of lymph nodes, ranging from beginning deposits to completely calcified ones, may be regarded as indelible stigmata of tuberculosis. From the viewpoint of latency the process represents a sclerotic termination of an active focus in the nodes. Obviously, caseous foci which are incompletely healed furnish the sources of metastases. Rabinowitsch (5), in 44 per cent of 34 calcified nodes, obtained positive inoculations, and Harbitz (6) demonstrated that 5 per cent of cases with tubercle bacilli in the lymph nodes were free from tubercle formation. Notwithstanding, the Research Committee of the National Tuberculosis Association disregards calcified lymph nodes at the root of the lung when lung disease is not present. "They are of no significance other than that of possible evidence of a healed inflammatory condition which may not be necessarily tuberculous. *This is a common finding in normal chest.*" Objections can be raised toward this statement on the ground that primary foci in the lung are frequently not detected even with the aid of X-ray. Furthermore, the Committee Report fails to come to any agreement as to what constitutes a "normal chest." Latent tuberculosis is not considered in their discussion apparently. From other sources there are studies which support our own viewpoint. As early as 1914 Wollstein and Bartlett (7) in a pathological study of 178 cases found that in about 10 per cent the bronchial lymph nodes were tuberculous despite the absence of infection in the lungs. Although Ghon might interpret this to mean that the lesions had been overlooked, the fact remains that it is difficult to make categorical antemortem statements about the absence of lung disease, in an attempt to minimize the significance of definite hilum shadows. Certainly there is no longer doubt that a pulmonic focus is accompanied by enlargement and caseation of hilum lymph nodes and of those in the bifurcation of the trachea and between the trachea and lungs. The studies of Opie and others have established this as a fact.

From the standpoint of roentgenological diagnosis a recent report of 4,000 cases by Crow (8) is also of interest in this connection. It was found that the posterior mediastinal lymph nodes were involved whenever pulmonary tuberculosis was present, even in early cases. The freedom from involvement of these nodes proved to be a negative sign of great value.

Hilum shadows may mean much or little, yet it is to be remembered that after clinical cure of tuberculosis physical as well as roentgenological signs of adenopathy do persist. While these shadows are not to be interpreted as signs of activity, they must be considered as a process in the course of evolution (9). To this may be added the general statement that an acute or chronic tuberculosis may involve either the lymph nodes or the lungs and, while the sequence in the tuberculous process can be shown clearly in X-ray studies, such as have been reported by Liss (10), the picture of early tuberculosis is not typical. Later stages show characteristic changes, but an early process cannot be distinguished from any other acute infection. Again, Belot (11) has stressed the importance of combined clinical history, symptomatology and roentgenological study in differential diagnosis between tuberculous and nontuberculous adenopathy.

Calcification of cervical lymph nodes: Calcification of cervical lymph nodes is not so rare as has been thought heretofore. Of greater importance in this connection is the unusual frequency of tuberculosis in such nodes. In tuberculous patients over five years of age more than 50 per cent in a series of 40 cases have been found positive by Hanford (12). When the X-ray failed to show increased density in the neck (19 cases), in 14 of these the cervical nodes proved to be tuberculous by microscopic examination or inoculation into guinea pigs. Out of a total of 200 juvenile patients who were examined 20 per cent had enlarged and calcified cervical lymph nodes. Our own observations suggest that these figures are not exaggerated. Wherever the portal of entry may be, we cannot subscribe, however, to the opinion of McEachern (13) who advocates extirpation of the faucial and pharyngeal tonsils in every case.

Primary Pulmonary Focus in Juvenile Tuberculosis

There is a dictum that no focus exists in the lung without tuberculosis of the tracheobronchial lymph nodes, or the reverse. Since this view was published by Küss (14), Albrecht (15) and Ghon (16), speculation

has been rife regarding the location of the primary focus and the direction taken by the infecting tubercle bacilli, that is, from hilum to parenchyma or from the pulmonary tissue inwards toward the hilum lymph nodes. If we side with Ghon, the infection marches to the centre from any part of the lung. The view of Harbitz (6) would assign the primary focus to the hilum nodes whence the infection spreads outward to the periphery.

Careful pathological studies of the infected lung in different stages of juvenile tuberculosis have given additional facts, yet without satisfaction to those who adhere to Ghon's theory or to that of the opposite school. Opie, in a small series of cases, demonstrated calcified or caseous tuberculous lesions in both lung substance and in lymphatic intrapulmonary lymph nodes near the hilum or in adjacent peribronchial and tracheal lymph nodes. In only one instance was a lesion found in lymphatic nodes when none was demonstrable in the substance of the lung. A consideration of the lymphogenous mode of infection would be incomplete without pathologico-anatomical studies. The arrangement of superficial and deep lymphatic vessels in the lung and the connections between subpleural lymphatics, the hilum nodes and those of the posterior mediastinum, suggest channels along which a tuberculous infection may travel.

From the precise studies of Méry and his associates (17), the probable course in tuberculosis of the tracheobronchial lymph nodes in children is in the nature of a subpleural infection with caseous involvement of the intrapulmonary nodes. Subpleural granulations develop in the subpleural lymphatic tissue, especially at the apex and base. A section of the pulmonary lobes shows the origin of the interlobar bronchi, and in the depth of this excavation near the hilum is found the hypertrophied interbronchial lymph node which is responsible for the inflammatory reaction in the interlobar pleura. Corresponding to the exit of this lymph node the induration of the lymphatic chains may be palpated. On the interlobar pleura tuberculous granulations are seen to coexist always with hilum lymph nodes. By cutting through the lung, peribronchial thickening can be noted accurately where the principal bronchus traverses the inferior lobe obliquely while giving off collateral branches to the other lobes at the level of the hilum. The hilum lymph nodes which are intrapulmonary appear thus in exact relation to the bronchovascular pedicle and are seen to be interbronchial. These may be congested or be the seat of gray or yellowish granulations, at times

completely caseous. Caseous adenitis is accompanied by adherent periadenitis to the parenchyma. These observations are significant from the standpoints of roentgen-ray interpretation and the importance of hilum adenopathy in diagnosing juvenile tuberculosis. Méry (18) believes that the bronchial lymph nodes carry tubercle bacilli to different parts of the body and, similarly, cause invasion of the lung by retrograde pathways.

Our own studies indicate that pulmonary foci are often overlooked on the X-ray film. These small zones of feathery infiltration are always found in the presence of indisputable hilum adenopathy. When, as may happen, clinical symptoms are negligible or absent entirely, despite a positive or suggestive history, these foci should establish a diagnosis and at the same time emphasize the relation between hilum lymph nodes and intrapulmonary foci in latent or active tuberculosis. On the other hand, since it has been possible to demonstrate primary foci in 66 per cent of autopsies on 347 children and, in 60 per cent of these, tuberculous changes in neighboring lymph nodes (19), the frequency of such correlation lends added significance to the presence of hilum adenopathy without visible pulmonary foci. In our series of juvenile patients foci were found in 46.5 per cent.

Pulmonary foci and X-ray findings: In this series of 145 cases pulmonary foci were found 67 times, a percentage of 46.5, and of these, 48 or 71.6 per cent showed calcification of the focus or the adjacent intrapulmonary lymph nodes.

The association of foci in the lung with calcification and enlargement of hilum or intrapulmonary lymph nodes was quite common. In a great number of instances these changes occurred in the corresponding lung. At other times parenchymal infiltration was observed in conjunction with these or as a process which supervened and came to notice only after subsequent X-ray films had been made. With the aid of lateral X-ray pictures it was possible to demonstrate readily the presence of mediastinal lymph-node changes which would have remained unrecognized otherwise. These areas of calcification and adenopathy, alone or in combination, went hand in hand with a definite clinical history and positive tuberculin reactions. In order that X-ray interpretations of juvenile tuberculosis be more complete, it seems advisable to take mediastinal as well as anteroposterior films of the chest.

Peribronchial tuberculosis, in the sense of definite involvement around

or adjacent to the bronchial ramifications, was demonstrable by X-ray in 5 per cent of the cases.

These observations might lead one to suspect that endogenous infection in children is not unusual. Superimposed infections are not likely to take a course which depends upon the development of resistance or immunity to a tuberculous process of remote origin.

A few typical roentgenograms will be described to illustrate the chief points.

A. Involvement of lymph nodes: Patient G. R., female, age 7 years, had a history of exposure to active pulmonary tuberculosis in the home. The child was irritable and slightly underweight. Tuberculin tests were strongly positive and the cervical lymph nodes were enlarged and indurated. *X-ray:* Numerous calcified bronchial lymph nodes on both sides, especially on the right, with a focus above the third rib, anteriorly, on the right side (fig. 1).

Patient L. B., female, age $8\frac{1}{2}$ years, gave a history of exposure to tuberculosis in the home. Tuberculin tests were uniformly positive. *X-ray:* Enlarged and calcified tracheobronchial lymph nodes, bilateral, with calcified lymph nodes and a focus at the level of the third rib on the right (fig. 2).

Patient A. H., female, age 10 years, gave a history of nervousness and lassitude for the past year. She tired easily and had a capricious appetite. Tuberculin tests were strongly positive, and were accompanied by lymphangitis with constitutional symptoms. *X-ray:* There were two large calcified lymph nodes in the upper part of the right hilum and smaller calcified nodules along the thickened bronchi extending outward from this region. On the left the bronchial thickening was most marked, especially at the base (fig. 3). A mediastinal film showed thickening in the upper posterior mediastinum with many enlarged and calcified lymph nodes (fig. 3a).

B. Peribronchial tuberculosis: This type of infection presented a variable picture which could be demonstrated satisfactorily in the X-ray film. The changes ranged from a simple thickening and slight beading of the bronchial markings, especially in the apices, to a definite peribronchial infiltration at the apex or base or both. At times this picture was augmented by small, irregular, calcified areas adjacent to

the bronchial prolongations, and, again, a thickened apical pleura would be found. In isolated instances the patients gave an early history of definite constitutional symptoms which were accompanied by cervical adenopathy and positive chest findings. An interesting follow-up of such a case is described below (E. W., age 8).

Patient H. E., female, age 3½ years, had measles one year ago, and was exposed for an indefinite period to pulmonary tuberculosis at home. The child had been underweight, restless, nervous, and had had no appetite. During the past year she had had occasional night-sweats. Tuberculin tests (Pirquet) were strongly positive and gave a lymphangitis reaction. *Chest examination* was negative except for slightly harsh breath-sounds close to the vertebral column. There was no paravertebral dulness. *X-ray:* Anteroposterior films, which were taken one month apart, showed large and partly calcified bronchial lymph nodes and extensive beading of the bronchial markings, especially in the apices (fig. 4). A diagnosis of peribronchial tuberculosis was made by the roentgenologist. Lateral chest films which were made, respectively, one and two months later, showed definitely enlarged bronchial and mediastinal lymph nodes (fig. 4a).

Patient E. W., female, age 8 years, was admitted to the clinic with the complaints of frequent "colds" and undernourishmemt. A sister died of tuberculous meningitis when this child was two years old. At the present time her mother has pulmonary tuberculosis of four years duration. Tuberculin reactions were positive in 1919, and repeated tests were positive this year. *Chest examinations:* (December 1919) There were harsh breath-sounds posteriorly. (February 1925) Over the left paravertebral trunk there were somewhat high-pitched breath-sounds, and at the right base there was slight dulness. *X-ray*: In

Description of Figures 1 to 4a

FIG. 1. Patient 7 years old. Bilateral calcified bronchial lymph nodes; intrapulmonary focus above third rib on right side. See page 133 of text.

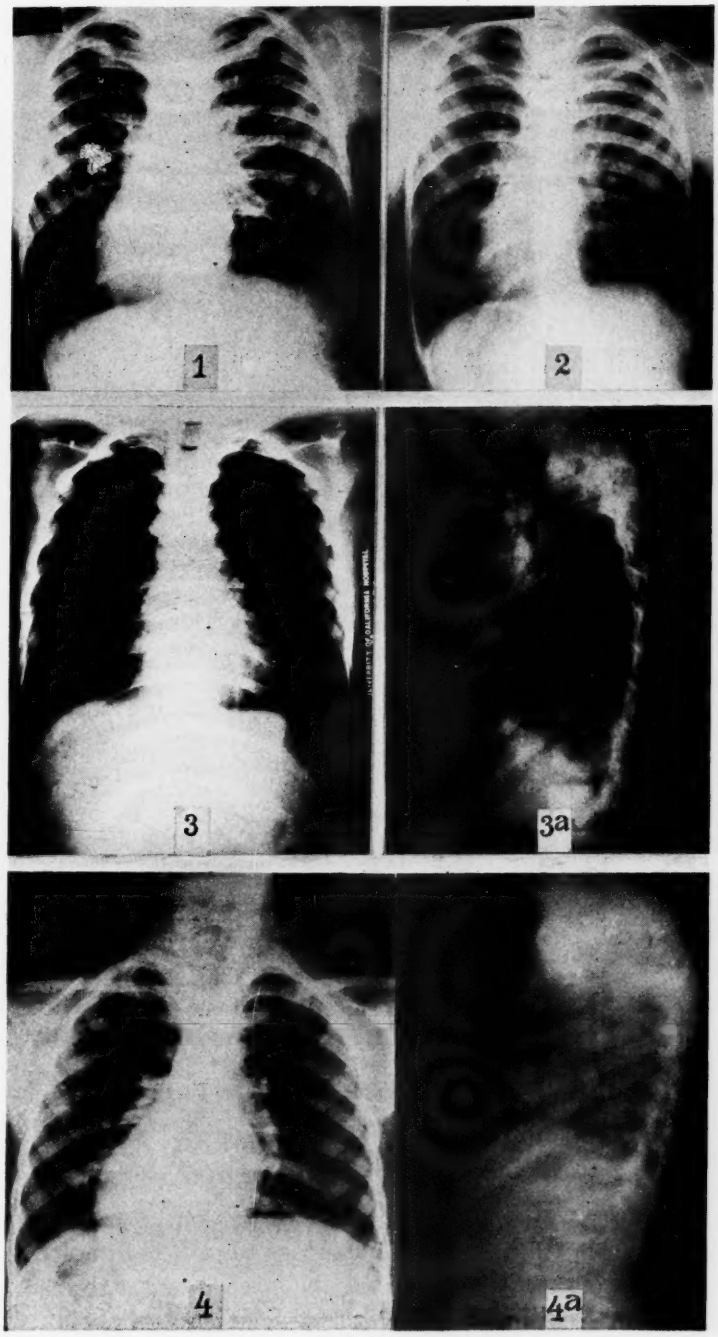
FIG. 2. Patient 8½ years old. Bilateral tracheobronchial adenopathy; calcified lymph nodes and focus at level of third rib on right. See page 133 of text.

FIG. 3. Patient 10 years old. Calcified lymph nodes at hilum on right; marked bronchial induration at left base. See page 133 of text.

FIG. 3a. Same patient as figure 3. Lateral film, showing posterior mediastinal thickening with calcified lymph nodes. See page 133 of text.

FIG. 4. Patient 3½ years old. Hilum adenopathy and extensive bronchial beading. See page 134 of text.

FIG. 4a. Same patient as figure 4. Lateral film, showing enlarged bronchial and mediastinal lymph nodes.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December, 1919, peribronchial thickening was present. In February, 1925, anteroposterior films showed large bronchial lymph nodes, thicken-

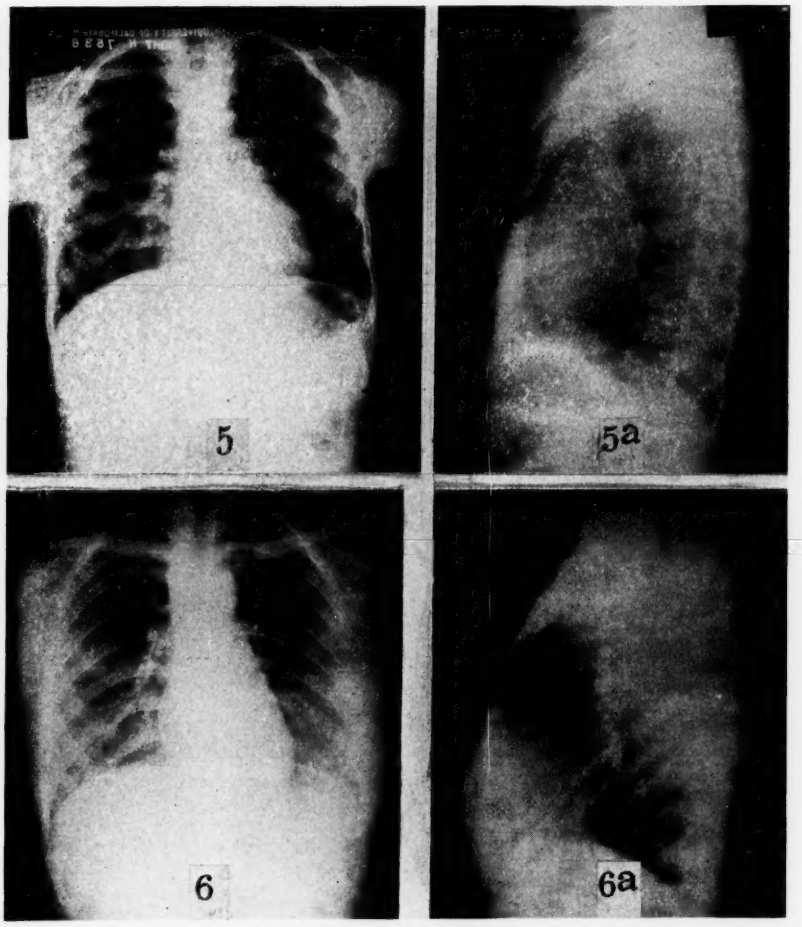

FIG. 5. Patient 8 years old. Peribronchial beading and calcification at hilum. See page 134 of text.

FIG. 5a. Same patient as figure 5. Calcified bronchial lymph nodes.

FIG. 6. Patient 16 years old. Enlarged bronchial lymph nodes, and peribronchial thickening in right upper lobe and toward right base. See page 137 of text.

FIG. 6a. Same patient as figure 6. Enlarged bronchial lymph nodes, and thickening in region of interlobar septum.

ing and beading in the bronchial markings toward both apices, and a small, irregular calcified area behind the sixth intercostal space on the right side. The diagnosis was peribronchial tuberculosis (fig. 5).

Lateral films showed calcified and enlarged bronchial lymph nodes with thickening of the bronchial terminations (fig. 5a).

Patient E. W., Chinese, female, age 16 years, had been exposed for the preceding five months to tuberculosis at home. She complained of lassitude. Tuberculin tests (Pirquet) were positive and reacted with a marked lymphangitis. *X-ray:* (September 1924) There were large bronchial lymph nodes and considerable peribronchial thickening in the right upper lobe and toward the right base (fig. 6). A lateral film (March 1925) showed large bronchial lymph nodes and thickening in the region of the interlobar septum on the right side (Fig. 6a).

The course of peribronchial tuberculosis in children can be followed for many years. In a number of cases we were able to study four and five children in the same family for as long as six to eight years. A typical example is given in some detail.

Patient W. K., male, age 18 years, was first admitted to the University of California Clinic as a contact in 1917 with a history of frequent night-sweats, loss of weight and lassitude. Swelling of the lymph nodes was present below each jaw. In May, 1917, tuberculin tests were slightly positive, and in August of the same year were definitely positive, at which time the *X-ray* film showed peribronchial thickening at both tops, and more marked on the right. In September the X-ray showed peribronchial thickening associated with cloudiness of the apices. The symptoms now were cough, night-sweats, poor appetite, and evening temperature which rose to 100° at times. *Chest examination* revealed dulness over the right apex posteriorly, with increased breath-sounds and distant breathing over the left apex, anteriorly and posteriorly. In January, 1918, the boy had a severe cough, and examination showed intrascapular dulness to the level of the fifth thoracic spine on the right and the third on the left, with a few medium râles. During the ensuing four years the patient's condition improved remarkably and he was put under observation. In February, 1922, he came to the clinic with a slight unproductive cough. Examination was negative and he had no other symptoms or signs. In February, 1923, tuberculin tests (Pirquet) were positive with bovine and human tuberculins, while intracutaneous tests were negative. X-ray on this date showed heavy hilum shadows with few small calcified lymph nodes. In November, 1924, examination of the chest was negative. Tuberculin tests were positive. X-ray showed heavy lung markings, but no foci. From February to April, 1925, the patient was still under observation, and he had no symptoms

or signs and the physical examination was negative. He is gaining weight steadily and feels strong.

C. Hilum tuberculosis: In fully 10 per cent of these cases pathological changes could not be distinguished readily from the types of old fibrotic lesions which occur in adult parenchymal tuberculosis. Involvement of the hilum lymph nodes was often associated with diffuse peribronchial thickening. Most commonly hilum changes were characterized by considerable calcification of the enlarged lymph nodes and an extension of calcified nodules toward the periphery of the lung (figs. 7 and 9). In other cases the areas of fibrosis and calcification ranged outward from the hilum and suggested the appearance of an extension of the tuberculous process to the parenchyma (fig. 8). Examples of these observations follow.

Patient O. G., female, age 8 years, was admitted to the clinic June, 1924, with indefinite complaints and failure to gain weight. There was a history of exposure to tuberculosis in the family. Her father died of tuberculosis in October of the same year. The child had measles at one and one-half years and dysentery at four, and no recent infections. Tuberculin tests (Pirquet) in October, 1924, were strongly positive and reacted with lymphangitis. *Chest examination* was negative. *X-ray* (November, 1924) showed considerable calcification in each hilum, most marked in the right, and large bronchial lymph nodes, with calcification and diffuse peribronchial thickening. There were several small calcified nodules, which extended far toward the periphery of the right base (fig. 7).

Patient L. O., female, age 8 years, complained of nervousness, fatigability and headache. The father has had pulmonary tuberculosis for the past four years. *Chest examination* revealed slight impairment of the percussion note at the right top anteriorly. There were no râles or other evidences of activity. *X-ray* showed an area of calcification and fibrosis extending outward from the upper end of the right hilum beneath the second rib anteriorly. This was interpreted by the roentgenologist as an old tuberculous focus, an extension of a hilum infection (fig. 8).

Patient R. G., female, age 12 years, gave a history of anemia, subnormal weight and enlarged lymph nodes in the neck. The father has had pulmonary tuberculosis since the child was 8 years old. On *physical examination* the lymph nodes of the neck were found greatly enlarged,

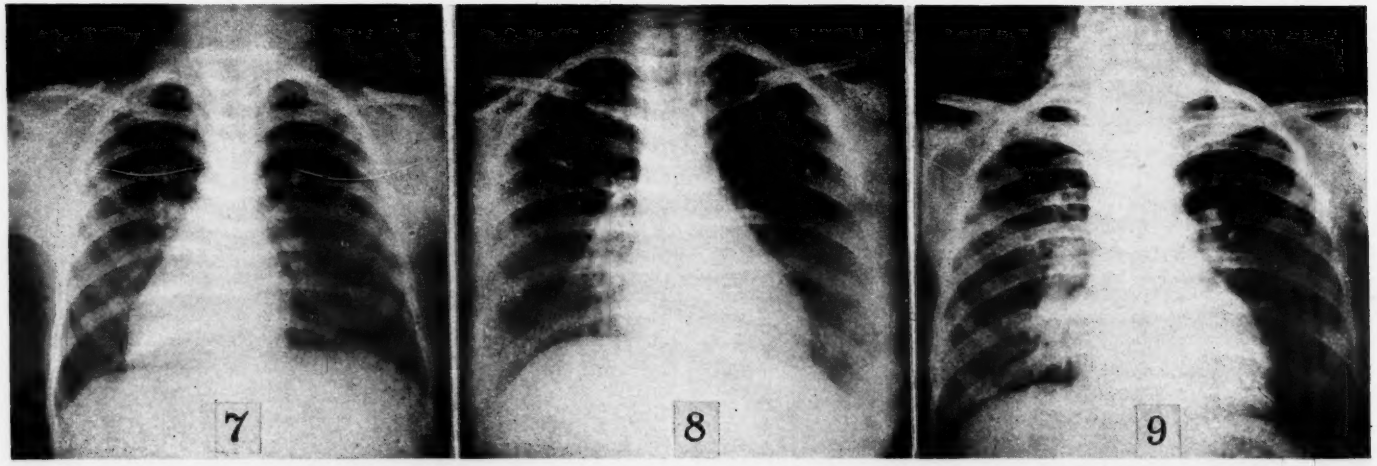

FIG. 7. Patient 8 years old. Bilateral calcification at hilum. Calcified bronchial lymph nodes and diffuse peribronchial thickening. Calcified nodules extending out into right base. See page 138 of text.

FIG. 8. Patient 8 years old. Calcification and fibrosis extending outward and upward from hilum on the right. See page 138 of text.

FIG. 9. Patient 12 years old. Marked calcification from hilum to periphery of lung. Calcified masses in neck. See page 138 of text.

and palpable along the course of the sternocleidomastoid muscle. The submental, anterior cervical and posterior auricular glands were uniformly enlarged. (Other possible causes of adenopathy were ruled out.) *Chest examination:* The breath-sounds were harsh and bronchovesicular in character, and there was slight dulness on the left side, especially in relation to the vertebral column. Tuberculin tests (Pirquet) were strongly positive with bovine tuberculins only. *X-ray* showed pronounced calcification and enlargement of the hilum lymph nodes with thickening and calcification extending to the periphery of the lung. The picture resembled old fibroid tuberculosis. Calcified masses in the neck were clearly shown in the X-ray film (fig. 9).

Juvenile tuberculosis and latency: The course of juvenile tuberculosis, while protracted as a rule, is generally favorable, depending upon the rigor of therapeutic measures. In most cases rest, proper diet and heliotherapy have resulted universally in restoring children to normal weight and strength, and in a cessation of all symptoms. The prognosis for the periods of adolescence and maturity depends obviously upon factors which would modify the course of any latent or arrested disease. The influence of certain infections, such as measles, influenza and pneumonia, in lighting up tuberculous foci is well shown in young patients. In adult life similar activating agents, whatever the manner in which these may operate to lower vitality and general resistance, must be borne in mind. Immunological phenomena teach that microorganisms may renew sallies at any time in the course of heterogenic infections. Experimentally, there is evidence to show that animals succumb to infections caused by latent pathogenic bacteria in "carriers." It is well known, for example, that mice may harbor the *B. murisepticus* with impunity until an infection with the pneumococcus is induced, when we find that the animals die with an overwhelming mouse typhoid infection instead of pneumonia. This fact is demonstrated repeatedly in different diseases, and has been pointed out in syphilis and trypanosome infections (20). Bearing upon the tuberculosis problem the studies of the Trudeau Sanatorium (21) are of interest. Over a period of 35 years it was shown in a selected group of patients who were discharged as apparently "cured" or "arrested," that the mortality was from two to three times the expected rate and the cause of death in from 60 to 70 per cent was tuberculosis. We have a cogent illustration here of the relapsing tendency of old foci. At all events, it seems that latency

and healed foci of tuberculosis in children do not offer a sharp line of demarcation for purposes of prognosis. In the adult there is pathological evidence that latent apical tuberculosis is accompanied invariably by preëxisting focal infections which have their origin in childhood (2).

TUBERCULIN REACTIONS

Skin tests were made by the cutaneous (Pirquet) and intracutaneous (Mantoux) methods, and in a small series of cases (15) a preliminary quantitative study was attempted with human and bovine tuberculins in each patient. Tuberculins were standardized and tested before use by the method whch has been described by Eagleton (22), assuring a constant potency of tuberculin measured by a "fixed" strain of tubercle bacilli in guinea pigs.

The results, in general, indicated that at least 75 per cent of all tests were positive with both Pirquet and Mantoux technique in each patient. Of the remainder there was approximately an equal division between the number who reacted more strongly to either one or the other, while about 1 per cent failed to react to both. This last group, however, gave definite reactions with specially prepared tuberculin derivatives.

The quantitative intracutaneous tests with both human and bovine tuberculins in dilutions of 1:1,000,000, 1:100,000 and 1:10,000 showed that certain patients will react more strongly or solely to bovine tuberculin in 73 per cent of the cases, while others react more violently to human tuberculin in 26 per cent of the cases, and in rare instances to the human type of tuberculin alone in 1 per cent of the cases. The explanation for these variations is not clear. The type of infection while conforming to the supposed bovine variety in most cases, fails to correlate satisfactorily in others, who, despite a similar picture, react only to human tuberculin. An interesting example to the point is that of a boy who was admitted at the age of 15 years with a tuberculous osteomyelitis, Pott's disease and lymph-node tuberculosis. Reactions to bovine tuberculin were positive at this time. Three years later repeated intracutaneous and cutaneous tests with both types of tuberculin were positive for human tuberculin only in dilutions as high as 1:1,000,000. These anomalies may find an explanation in the alteration of resistance to the infection with possible "extinction" phenomena due to the development of immune bodies. As an alternative it may be assumed that there is only one type of tuberculosis with protean

manifestations which are dependent upon the age of the person as well as upon the site of infection. The problem has not been settled and requires a selected group of cases in which tuberculin reactions can be studied with the aid of a quantitative comparative technique.

Lymphangitis following Pirquet tests has been described in an earlier publication (23). In the present series, which contains additional material, the phenomenon occurred in three patients whose history and X-ray findings were positive. The lymphangitis reaction developed with the bovine tuberculin in two cases and with human tuberculin in another. Analysis of these histories confirms the observations which were made previously, and are appended in brief.

Patient H. E., female, age 3½ years, gave a history of nervousness, restlessness, poor appetite and undernourishment, with occasional night-sweats during the past year. She had measles one year ago. *Physical examination:* There was bilateral cervical adenopathy, and chest findings were negative except for slightly harsh breath-sounds adjacent to the vertebral column. *Tuberculin reactions:* The Pirquet test was positive with human tuberculin, with the development of an intense reaction after 48 hours. An areola 3 x 3 cm. and a central zone of 1.5 x 1 cm. were accompanied by a lymphangitis streak 6 cm. long and 1 cm. wide. The lesion at its centre was umbilicated, indurated and erythematous. The reaction faded on the fifth day. *X-ray:* Anteroposterior films showed large, partly calcified bronchial lymph nodes and extensive beading of the markings, especially in the apices. The diagnosis was peribronchial tuberculosis. Lateral films which were taken on three subsequent occasions demonstrated enlarged and calcified mediastinal and bronchial lymph nodes.

Patient R. J., female, age 14 years, whose father and brother died of tuberculous meningitis seven years ago, presented vague symptoms only, such as capricious appetite, frequent "colds" and irritability. The *chest examination* was negative. The *X-ray* showed diffuse thickening of the bronchial markings, with pronounced enlargement of the bronchial lymph nodes. *Tuberculin reactions:* The Pirquet test with bovine and human tuberculins was positive. A lymphangitis streak, 2 cm. wide and extending as far as the axilla, developed at the site of the bovine tuberculin test, which covered an area of 2.5 x 2 cm. The human tuberculin was positive over an area of 1.5 x 1.5 cm.

Patient G. J., female, age 9 years, a sister of the preceding patient, has had no symptoms other than persistent discharges and pain in one

ear. The *chest examination* was negative. The *X-ray* showed large bronchial lymph nodes and fibrous thickening behind the second right interspace. *Tuberculin reactions:* Tests with bovine tuberculin were positive over an area of 1.5 x 1 cm., accompanied by a streak of lymphangitis 6.5 cm. long and 1 cm. wide. Human tuberculin reacted positively over an area of 1.5 x 1 cm.

Recent studies (23) have suggested the relation between tuberculosis of the lymphatic system and the diagnostic tuberculin test in children. Sensitization of the lymph channels and the response to tuberculin apparently go hand in hand. In juvenile tuberculosis studies with tuberculin find an important place because the infection is essentially lymphatic in type. Too much emphasis cannot be placed on a thorough examination of lymph nodes in patients with adenopathy, or in those who are below par. Fraser (24), Philip (25), and Hanford (12), in detailed pathological studies of the important groups of lymph nodes, have found positive evidence of tuberculosis. Of particular interest is Hanford's series of 200 cases, in which the frequency of tuberculosis in the cervical lymph nodes was such as to warrant revision of the conservative ideas regarding adenopathy of these groups of nodes.

Human and bovine tuberculin in differentiation of type of infection: It has been stated generally that neither the cutaneous nor the intracutaneous injection of both types of tuberculin shows the kind of tuberculous infection when tests are applied simultaneously. Observations by Nehring (26), for example, indicate that a difference in degree of reaction in the first test may be reversed in a subsequent one; Synwoldt (27), on the other hand, believes that an incipient infection tends to react to the more susceptible bovine tuberculin at a time when the patient is insusceptible to human tuberculin. Yet these same juvenile patients show at different times a predilection for either bovine or human tuberculins. Discrepancies of this nature lead one to believe that there are many factors which complicate the picture, such as differences in toxicity and potency of tuberculins and the general susceptibility or hypersensitiveness of the patient. If we incline toward the view of Bernheim-Carrer (28), the best and most uniform reactions will be found in latent or in early stages of tuberculosis, and the irregularities in the tests will be due less to technique and the kind of tuberculin used than to the type of clinical material. In his series of 143 positive cases, 59 per cent showed no difference, while 41 per cent gave more pronounced reactions, or else positive results with one type of tuberculin only.

These figures differ from our own which have been mentioned on page 141. In the absence of transitory causes of anergy, repeatedly negative reactions certainly should exclude tuberculosis, yet a positive test in the presence of adenopathies once more raises the question as to how the test is to be interpreted. Is the reaction, according to Marfan (29) and others, merely an indication that living tubercle bacilli are present somewhere, irrespective of activity or inactivity of a lesion, or are most of the adenopathies nontuberculous? Génévrier (30), in a recent paper, does not come to any definite conclusion, while stating that measles and pertussis cause adenopathy and that enlarged lymph nodes may frequently become tuberculized. Observations of divergent character may be multiplied in the studies of Veeder and Johnson (31), Hamburger (32), Stern and Schultz (33), Rüscher (34), Dietl (35), Wahl and Gerstenberger (36), Drolet (37), and many others.

These conflicting opinions regarding the value of differential tuberculin tests may be explained perhaps by some deficiency in experimental procedure. It is noteworthy that for the most part the tuberculin tests have been performed by the Pirquet cutaneous method. When a more accurate intracutaneous technique has been described, either dilutions have not been used at all, or at best these have not been great enough to serve as criteria for determining actual measurable differences. Conditions being equal, a series of highly graduated dilutions, injected simultaneously, should give quantitative comparable results with different kinds of tuberculin when these have been standardized previously. If there be any differential diagnostic value in these, such a method might be expected to answer the question.

The writer believes that the value of a positive tuberculin test in the presence of adenopathy may be considered from yet another angle. The self-same diseases of childhood which may cause nonspecific adenopathy are known to "extinguish" tuberculin reactions. Hence, a positive tuberculin test under these conditions should leave no room for doubtful interpretation.

From previous studies and those which are now in progress, the evidence favors the view that the interpretation of tuberculin reactions depends upon the kind of tuberculin used, and that tests will be of value only when made with a derivative containing the active principle. It has been shown (38) that the intensity of a reaction and the range of specificity are immunochemical functions of the tuberculin. Fur-

ther work with tuberculin derivatives in this direction is to be published in the near future.

SUMMARY AND CONCLUSIONS

One hundred and forty-five cases of juvenile tuberculosis have been studied with regard to history, age-groups, symptomatology, physical and X-ray findings, and tuberculin reactions. The following deductions are drawn from observations which have been made on a series of patients who, without exception, had been exposed to tuberculosis:

The majority of these patients were under observation for a period of 1 to 3 years, and approximately 25 per cent of the group for 6 months to 1 year, while about 10 per cent have been studied over a period of 6 to 8 years.

Regardless of the degree of exposure to infection, the greatest number of cases, 72 per cent, fell in the age-group of 1–9 years and of which 58.6 per cent were between the ages of 5 and 9 years.

Analysis of the different symptom-complexes which occurred showed that it is not possible to make unequivocal statements regarding a symptom-group which may be called pathognomonic of tuberculosis. The diagnostic importance of any one symptom or of a combination of symptoms could not be measured in view of the elusiveness of these. The evaluation of single or grouped symptoms became relatively accurate when these were interpreted in relation to X-ray and tuberculin studies.

Physical examination yielded information which was of slight value except in patients who were frankly tuberculous. D'Espine's sign was not found to be diagnostic and was not correlated with adenopathic changes.

X-ray studies furnished probably the most important and significant data in this investigation when used in conjunction with other observations. It would appear that *pulmonary foci are frequently overlooked.* There is evidence here to show that indisputable hilum adenopathy associated with a pulmonary focus and a positive or suggestive clinical history, even when symptoms are negligible or absent, warrants a diagnosis of tuberculosis. There appears to be a definite relation between hilum nodes and intrapulmonary foci in latent tuberculosis of children. *Pulmonary foci were found in 46.5 per cent of the patients* in this study, and of these 71.6 per cent showed calcification of the focus or of the ad-

jacent intrapulmonary lymph nodes. Parenchymal infiltration occurred at times, in conjunction with such changes or as a supervening process which came to notice after subsequent X-ray studies had been made. *Peribronchial tuberculosis of the nature of definite pathological changes or adjacent to bronchial ramifications was demonstrated by the X-ray in 5 per cent of the cases.* The changes ranged from simple thickening and slight beading of the bronchial markings, particularly in the apices, to a definite peribronchial infiltration at the apex or base or both. At times, small irregular, calcified areas adjacent to the bronchial terminations augmented the usual picture. *Mediastinal adenopathy of clinical significance could be shown readily with the aid of lateral X-ray films.* These were found to be indubitably valuable in recognizing lymph-node changes which remain undiscovered otherwise.

Hilum tuberculosis was characterized chiefly by considerable calcification of enlarged lymph nodes, with an extension of calcified nodules toward the periphery of the lung. In some instances there were localized fibrosis and calcification, which ranged outward as an extension of a tuberculous process to the parenchyma. In 10 per cent of the cases the pathological changes were hardly distinguishable from old fibrotic lesions which occur in adults.

Tuberculin reactions were studied with a view to determine (1) the degree of correlation between positive tests and probable tuberculous infection, (2) the frequency of positive results when different techniques (Pirquet and Mantoux) were employed and (3) the differences noted with the use of bovine and human tuberculins when applied simultaneously in the same patient. The results indicated that there was a *complete correlation between positive tuberculin tests and clinical and X-ray findings which warranted the diagnosis of latent or active tuberculosis.* Seventy-five per cent of all tests were positive for both cutaneous (Pirquet) and intracutaneous (Mantoux) methods. Of the remainder, the number which reacted more strongly to one or to the other was approximately equally divided, while about 1 per cent reacted to neither. In this last group, however, definite reactions could be elicited when tuberculin derivatives were used. *Quantitative intracutaneous tests with bovine and human tuberculins demonstrated the following:* 73 per cent reacted more violently or solely to bovine tuberculin; 26 per cent reacted more strongly to human tuberculin; and 1 per cent reacted to human tuberculin only. Lymphangitis accompanying tuberculin tests

occurred in three additional cases, and confirmed the observations which have been reported in an earlier publication.

A study of the tuberculin reactions in this series and in previous experimental work leads to the conclusion that an interpretation of the test depends upon the kind of tuberculin used, and that a test is of value only when made with derivatives of tuberculin which contain an active principle that is as yet not clearly determined. *Quantitative comparable results can be obtained by the use of highly graduated dilutions of different types of tuberculin when these have been standardized previously.* The intensity and range of specificity of the reaction seem to be immunochemical functions of the tuberculin. Further work in this direction is now being carried on and is to be the subject of a future report.

The writer believes that the value of a positive tuberculin test in the presence of adenopathy cannot be doubted if it be remembered that the self-same diseases of childhood which may cause nonspecific adenopathy are known to "extinguish" tuberculin reactions.

Latent tuberculosis in childhood ought to be considered in the light of known immunological facts which teach that heterogenic infections can and do light up preëxisting foci. A latent or "carrier" state exists in tuberculosis as in other diseases, notably malaria, typhoid, trypanosomiasis and syphilis.

For the purposes of prognosis in children, it appears from the present study that latency and healed foci of tuberculosis do not offer a sharp line of demarcation. Moreover, endogenous infections in children are not infrequent, inasmuch as a superimposed infection must obviously take a course which is modified by the development of resistance or immunity. In children it is not a question of a remote tuberculosis, and such alterations are hardly likely to occur.

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EXTRAPLEURAL THORACOPLASTY

A Report of 12 Cases[1]

LEROY S. PETERS[2]

It has always been my opinion that artificial pneumothorax in properly selected cases is the best treatment for many patients with pulmonary tuberculosis; and this is still my opinion, even after the introduction of more radical lung surgery. I cannot agree with chest surgeons who believe thoracoplasty is preferable to pneumothorax because of the lasting compression, and because the patient is not subjected to a long-drawn-out form of treatment, such as must necessarily be practised in making refills. These same chest surgeons deplore the complications of induced pneumothorax, which exist mostly in the earlier literature on the subject, and which are in reality a figment of the imagination when the work is done by an experienced operator.

The world is still filled with unexperienced operators, or, what is worse, men who apparently never learn; but that same criticism can probably in the future be directed at chest surgeons, for I venture to predict that soon every man who follows surgery, not because of any inherent ability, but because he sees an easy way to augment his bank account, will pose as a chest surgeon. If this prophecy proves true, a severe blow will be dealt pulmonary surgery, from which it will take years to recover. It takes a far better surgeon to perform a thoracoplasty than to remove an appendix, just as much so as it takes a far better chest specialist to select cases and carry on a course of pneumothorax treatment, than to puncture unsuspecting humanity with various hypodermic cures.

Let us hope I have made myself perfectly understood: first, artificial pneumothorax is to be preferred to thoracoplasty; second, thoracoplasty should be attempted on all patients on whom a pneumothorax cannot be performed because of existing adhesions—provided the physical condition is fairly good and provided the clinician did not attempt pneumothorax as a last resort, gambling on the possibility, but not probability, of the

[1] Read at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

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contralateral lung standing the strain. The choice of cases for rib resection must be much more closely hewn than for pneumothorax. The contralateral lung must be practically normal, or must have but a minimal lesion located in the extreme upper fields. Once a thoracoplasty is done, it cannot be undone, which, of course, is not true for a pneumothorax. Hence, in the latter the gamble can be greater.

The better the physical condition of the patient, the better the chance for success. A tuberculous individual is a poor surgical risk at best, and I have no patience with the clinician or surgeon who takes long chances merely to gratify the whim of a patient, or to add another number to his series. For that reason, choose patients in whom you have reasonable hopes of completing the operation. Fibroid lungs with a marked amount of contraction are best, for, as Archibald points out, this in itself is a sign of resistance and there is less chance of a sinking in of the lung, thereby causing paradoxical respiration.

Chest surgeons must be developed locally; otherwise, comparatively few patients can avail themselves of thoracoplasty, even when it has been advised. We have found it possible to develop two good men in Albuquerque, men who are doing excellent work in chest surgery as the appended results will show. Close coöperation between surgeon and clinician is an essential factor for success. Without such coalition little can be expected in the way of end-results.

At first we did a one-stage operation with apparently no ill effects; but since reading Archibald's last paper I was surprised to find that the only one-stage operation he had ever done was on a patient of mine. Lately we are doing the operation in two stages, and I believe there is much less discomfort and, no doubt, less danger to the patient. However, one operative death occurred in a patient in whom we had resected but five ribs and who died a few days later from heart failure. However, this patient was not a good risk, but he had been bleeding for five months. I had been unsuccessful in producing a pneumothorax and hesitated on a thoracoplasty because of the opposite lung. This, however, cleared up remarkably during the five months in bed and made him a fair risk. The bleeding stopped after the first-stage operation was completed. Had we resected shorter lengths of rib in this case the outcome might have been different. When little fibrosis is present less rib must be cut than when the lung is hard from a fibrotic condition.

As to choice of anaesthetic, operators differ. We tried, at first, gas-oxygen; but now we are doing all operations under local anaesthesia

unless the patient complains too much. This does away with possible aspiration, and so far we have had no ill effects from the 1 per cent novocaine.

My reason for reporting so small a series is solely to augment general statistics on the subject. All of us have but few cases, with the exception of men doing chest surgery in large centres of population, and even their series cannot be called large. If every one would publish his cases in a reasonable time we could gather statistics as to operative mortality and end-results that would be of great value in forming a final estimate of the operation.

Case Reports

Case 1: This patient had been under my care for three years previous to the thoracoplasty; pneumothorax could not be done because of adhesions. In the summer of 1921 I sent her to Saranac Lake, and wrote her people to keep her at home, because I felt certain she could not survive another winter. Dr. Brown suggested a thoracoplasty, which was done by Dr. Lilienthal October 8, 1921. She left the hospital in three weeks, was home two months and gained 15 pounds. She returned to Albuquerque in December, 1921, and home in May, 1922. Operated on in December, 1922, for ruptured gangrenous appendix, and in two weeks operated on again for abdominal abscess. She left the hospital in four weeks. To Miami, Florida, in January, 1923; another abscess. Home April, 1923. Diphtheria May 14, 1923; well in two weeks, but left with some bronchitis. Florida for the winter of 1923–1924. Milk diet three months and gained 18 pounds. At present is well and last examination this winter showed "dry" chest, and very little sputum, negative for tubercle bacilli.

Case 2: Admitted December 10, 1922; female; age 30. Fibroid tuberculosis entire left lung with large cavity at apex. Slight fibrosis right apex. Attempted pneumothorax May, 1923, but could not find a pleural space. From then on she ran continuous fever, averaging 100° daily. Dr. P. G. Cornish, Jr., did a thoracoplasty under local anaesthesia March 18, 1924, resecting from 11th to 1st ribs, taking out five inches of 11th and 1½ inches of 1st rib. Admitted to hospital March 18, 1924; discharged April 7, 1924. Temperature fell to normal on discharge, but gradually rose so that through the following summer she was running an average of 100°. On fluoroscopic examinaton the lower lobe was seen to be expanding; so on August 11, 1924, a portion of the 12th rib was removed. The 11th was found fused to the spine, and a resection of 1½ inches was done. Recovery uneventful, and patient was discharged August 21, 1924. Weight before first operation was 103; weight

May 8, 1925, is 125. Sputum slight in amount and free from tubercle bacilli; and patient has been working in a "beauty parlor" for three months.

Case 3: Admitted June 20, 1923; female; age 28. Fibroid tuberculosis entire left lung with multiple cavitations from apex to 7th dorsal spine; remainder of lung very dense. Some fibrosis right upper with few râles after cough. Patient had artificial pneumothorax attempted June, 1923, with no result, and had been running an average temperature of 103° daily from January, 1923, to time of operation. Dr. Archibald was called from Montreal; and, after seeing the patient, offered little hope of her recovery. Her condition was grave and she certainly was a poor surgical risk. On July 27, 1923, a thoracoplasty was done under gas-oxygen anaesthesia, and portions of ribs from the 10th to 1st inclusive were removed, 4½ inches to 1 inch in one stage. Patient's condition fairly good through operation, and temperature never ran higher than 100° after the resection. On July 28th blood transfusion done. Patient gradually regained strength, and left hospital for Arizona, September 23, 1923. For six months her temperature remained normal; then followed what she called influenza; and every two or three months afterward she would have a lessening of cough and sputum with high fever, due, I think, to poor drainage. This would last a few days until fair drainage would be established, when these symptoms would abate and all be normal again. Last attack occurred in October, 1924, and she has been perfectly well since. At present she has very little sputum (before operation about 500 cc. in 24 hours); slight cough and leads a normal life. Sputum negative. Weight, gained 29 pounds.

Case 4: Admitted November 29, 1921; age 35; female. Active infiltration right upper. Did pneumothorax March, 1922, because of gradual extension of disease throughout entire right lung. Patient did well, and was allowed to return home summer of 1924. Owing to the fact that she had to go a long distance for refills, the lung gradually expanded and on her return was well out. Repeated attempts at compression failed to collapse the lung, and a thoracoplasty in one stage was done September 3, 1924. Under local anaesthesia, portions of the 10th to 1st ribs were resected by Dr. W. R. Lovelace. The patient was in shock for 24 hours, and for a time it looked as though she could not live. However, she survived, and made an uneventful recovery. She was able to be up in wheel-chair October 8, 1924, and was walking short distances by October 17, 1924. By January, 1925, she was able to enjoy auto rides, and be up and about the greater part of the time. She left in May for a visit home this summer.

Case 5: Admitted July 21, 1924; age 29; female. This patient had been under observation eighteen months prior to admission to sanatorium. The

right lung showed fibrous apex. *Left:* general fibrosis, caseous tuberculosis. Attempted pneumothorax, but found adhesions. Thoracoplasty was done in one stage under local anaesthesia August 6, 1924, by Dr. W. R. Lovelace. Removed 10th to 1st ribs inclusive. Condition good throughout entire operation. Left hospital September 10, 1924, and has improved gradually ever since. Sputum free from tubercle bacilli. Consider the condition at present arrested. She is now East on a visit.

Case 6: Admitted July 19, 1924; age 43; male. This patient had been under my observation for four years with intermittent attacks of fever reaching 103° to 104° following a blockage of drainage from large cavity in right upper lobe. As soon as drainage was established, the fever would drop, and he would feel well for three or four weeks, only to repeat the reaction. Pneumothorax was attempted in August, 1924, but adhesions prevented a collapse. The cavity seemed to be the cause of all his trouble, lower and middle right and entire left lung being very free of tuberculosis. For this reason it was decided that, if possible, we would resect from the 1st to the 8th ribs inclusive, hoping to compress the cavity and leave the lower portion of the lung free. The first operation was done under local anaesthesia in September, 1924, by Dr. W. R. Lovelace, but this proved a failure. The cavity did not compress. On December 18, 1924, a second operation was done under local anaesthesia, removing 3 inches of 8th and 6 inches of 9th and 10th ribs. The result was excellent. For past three months patient has been free from fever and is able to lead a normal life within limits.

Case 7: Admitted April 5, 1923; female; age 24. Fibroid tuberculosis of entire left lung with cavity second interspace. This patient ran high fever, 101° to 102° daily, and a pneumothorax was done at once. A partial compression was obtained; fluid formed, and in spite of careful watching the lung expanded into fluid and could never be collapsed. Later we advised a thoracoplasty, which was done in one stage by Dr. P. G. Cornish, Jr., February 2, 1924, under gas-oxygen anaesthesia. Portions of ribs from 10th to 1st inclusive removed (5 to 1½ inches). Entire lung hard and firm except for some softening over lower lobe. Although this patient had no abdominal symptoms prior to operation, a few days following the thoracoplasty she began to complain of pain in region of the appendix, and by the sixth day there were some distention and loose bowel movements, which kept up daily throughout the course of the disease. On February 20, 1924, an exploratory operation was done, which disclosed a tuberculous caecum and a generalized peritoneal tuberculosis, with abdomen filled with fluid. The incision was closed, and the patient died March 3, 1924.

Case 8: Admitted December 13, 1921; discharged September 3, 1921, arrested. Readmitted August 22, 1924, far advanced. Dense fibrosis entire

left lung. Large cavity central left; right clear. Marked dyspnoea. Attempted pneumothorax in October, 1924, but was unsuccessful because of adhesions. First stage operation: thoracoplasty done February 21, 1925, under local anaesthesia by Dr. P. G. Cornish, Jr.; 11th to 7th ribs, inclusive, resected, sections varying from 4 to 3 inches. Lower lung found soft and some sinking on respiration. Recovery uneventful and second operation done March 16, 1925, under local anaesthesia, and sections of 6th to 1st ribs, inclusive, removed. Patient now running normal temperature and feeling very much improved, but it is too soon to predict end-result. It is interesting to note that, following the second operation, the dyspnoea which was formerly so troublesome has entirely disappeared.

Case 9: Admitted May 17, 1922; age 27; male; far advanced. Fibroid left lung, very dense with large cavity. Infiltration right upper. Attempted pneumothorax because of haemorrhages, October, 1924, but was unsuccessful because of adhesions. On May 1, 1925, partial thoracoplasty done by Dr. W. R. Lovelace, who removed, under local anaesthesia, 10th to 4th ribs inclusive. This patient felt so well throughout entire operation that he asked to have remaining three ribs removed. On May 15, 1925, 3rd to 1st ribs removed under local anaesthesia. Patient doing well in every way, but it is too soon to predict end-results.

Case 10: Admitted November 25, 1924; age 36; female. Fibroid tuberculosis left lung with cavity at apex. Remainder of lung very dense. Some fibrosis right upper with few râles after cough. This patient ran low-grade fever daily with excessive cough and sputum. Attempted pneumothorax with no result because of adhesions. On March 25, 1925, Dr. P. G. Cornish, Jr., did a two-stage thoracoplasty under local anaesthesia, resecting portions of 11th to 6th ribs inclusive, removing 5 to 4 inches. Area of lung under resection very soft. On April 28, 1925, under local anaesthesia, resected portions of 5th to 1st ribs inclusive (4 to 2 inches removed.) Patient stood both operations well, but it is too soon to predict results. She left the hospital May 20th to live in sanatorium.

Case 11: Admitted February 12, 1924; age 27; male. Scattered fibrosis left with cavity in apex. Very little contraction. Infiltration right upper and middle lobes. Attempted pneumothorax because of bleeding, with partial success through March and April, 1924, but could never get a real compression; so abandoned the treatment. In November, 1924, began to have small haemorrhages, which continued for weeks. By bed-rest, because of bleeding, the right lung became practically clear, and the first stage of a thoracoplasty was done by Dr. W. R. Lovelace March 9, 1925, under local anaesthesia. The bleeding immediately stopped, but the patient died of cardiac failure, March 19, 1925, ten days after the operation. This patient showed very

little dense fibrosis, with almost no contraction, and the lower portion of the lung was very soft, which, no doubt, explains the cardiac failure.

Case 12: Admitted March 19, 1925; age 28; female. Fibroid caseous tuberculosis entire right lung, with very little retraction. Some honeycombing. *Left lung:* some infiltration apex, but apparently inactive. Patient poor surgical risk; temperature 103° daily, pulse 130, and generally very ill. Advised thoracoplasty as last resort. First operation done by Dr. W. R. Lovelace under gas-oxygen anaesthesia, March 25, 1925, but could resect only four ribs due to unfavorable condition of patient. Two weeks later did second operation under gas-oxygen anaesthesia, resecting six more ribs through first. Patient came through this operation better than the first attempt, but died of cardiac failure eight days later.

This patient had pneumothorax attempted in the Fall of 1924, and kept up until she was admitted to St. Joseph's, but at no time in my opinion was the compression effective. Only a few small pockets of air could be seen on the negative. The case showed a condition similar to case 7: no dense fibrosis and lung too soft.

TABULAR SUMMARY OF RESULTS

Quiescent refers to patients with sputum free from tubercle bacilli and patients who are able to lead a normal life. *Improved* refers to patients who show tubercle bacilli, but are on exercise and better than before operation. I do not feel that I can classify any as arrested until a longer interval intervenes.

Leaving out the patient who died of general peritonitis, the deaths due directly to operation were two, or a percentage of $16\frac{2}{3}$.

CASE	QUIESCENT	IMPROVED	TOO SOON TO CLASSIFY	DEAD
1	Yes	0	0	0
2	Yes	0	0	0
3	Yes	0	0	0
4	0	Yes	0	0
5	Yes	0	0	0
6	0	Yes	0	0
7	0	0	0	Yes
8	0	0	Yes	0
9	0	0	Yes	0
10	0	0	Yes	0
11	0	0	0	Yes
12	0	0	0	Yes
Per cent.....	$33\frac{1}{3}$	$16\frac{2}{3}$	25	25

ARTIFICIAL PNEUMOTHORAX

A Report of Ten Years' Experience with 207 Cases

SAMUEL SWEZEY AND ALEXANDER SCHONBAR

Notwithstanding the fact that very much has been written on artificial pneumothorax, quite a few of its problems are far from being settled. There is still a strong minority that is skeptical as to its efficacy. The question of how early the treatment should be administered is still a subject of vigorous debate. There is also a difference of opinion as to the method of giving the inflations, the form of anesthesia, the frequency of the inflations, the quantities of air and the optimum pressures for each inflation, and the indications and contraindications for the treatment. We therefore feel that additional information on this subject will not be superfluous.

This represents a report of 207 cases of artificial pneumothorax treated at the National Jewish Hospital for Consumptives, Denver, Colorado, for a period of over ten years, during which time different methods of technique, as well as different types of cases, have been tried out, and the clinical observations, including a great deal of radiography and laboratory tests, have been carefully recorded. The fact that patients are allowed to stay in the National Jewish Hospital for eighteen months is of importance, as it affords an opportunity to observe cases for long periods of time under sanatorium regimen. Of the 207 cases 205 had definite pulmonary tuberculosis while two had bronchiectasis without any demonstrable phthisical lesions. Of the 205 cases, 191, or approximately 93.5 per cent, were far advanced, and 14, or approximately 6.5 per cent, were moderately advanced. No incipient cases were selected for treatment. No case was subjected to the treatment unless several months of usual sanatorium care failed to check the progression of the disease.

CHARACTER OF COLLAPSE

Since the object of artificial pneumothorax is to approximate, as much as possible, the walls of cavities, and to immobilize the active foci of the lung, we have divided our cases, from the standpoint of the character

of the collapse, into two main groups. In the first group are those who enjoyed successful collapse, in which the treatment was responsible for the compression of the major part of the active lesion in the lung regardless of what effect it had on the patient clinically. In the second are the patients with partial collapse, in which on account of adhesions the active lesions were affected by the pneumothorax treatment only slightly or not at all. Thus, in this group there are included many cases in which the major portion of the lung was collapsed, but that part of the lung which was most active was not affected by the treatment on account of adhesions. When intrapleural conditions prevented the introduction of air, or when adhesions caused an early obliteration of an established pneumothorax, the cases were classified as failures. There were altogether 79 patients, or approximately 38 per cent of the total series of 207, who sustained a successful collapse, and 75, or about 36 per cent, a partial collapse. In 53 cases, or 26 per cent of the total number, the treatment proved a failure. This made a ratio of 3:3:2. A left-sided pneumothorax was effected in 45 of the 79 successfully collapsed cases, and in 37 of the 75 partially collapsed cases. Of the 79 patients with a successful collapse, 72, or 91 per cent, were far advanced, and 9, or 9 per cent, moderately advanced. There were 71, or 95 per cent of the partially collapsed cases, who were far advanced, and 4, or 5 per cent, moderately advanced. Regarding the failures, 83 per cent were far advanced, and 17 per cent moderately advanced.

Eighteen of the 72 patients with a successful collapse are still under observation in the hospital wards. Concerning the remaining 54, 27, or 50 per cent, were discharged from the hospital as greatly improved, and 13, or 24 per cent, as improved. In 5 cases, or 9 per cent, the condition remained unchanged; in 3, or 5 per cent, it became worse, while 6, or 11 per cent, died.

Of the 53 failures, 6 were greatly improved, 16 improved and 5 unimproved. In 3 the condition became worse while 16 died. The remaining 8 are under observation in the hospital wards.

COMPLICATIONS PRESENT BEFORE COMMENCEMENT OF TREATMENT

The experience of others, as well as our own observations, has taught us that serious tuberculous complications invariably serve as contraindications to the administration of collapse therapy. Consequently, strenuous efforts have been made to adhere closely to the policy of elim-

inating cases with serious complications as unsuitable for treatment. However, a deviation from this rule was occasionally made when induced pneumothorax was administered either as an emergency measure, for instance, to check severe haemorrhages, or when we yielded to the temptation of testing the treatment on apparently hopeless cases.

Thus, two of our cases with a well defined tuberculous enteritis received the treatment for the control of haemoptysis. Because of pleural adhesions it proved unsuccessful in one, while a successful collapse was effected in the other one. The results in both instances were fatal.

In two other cases with a diagnosis of questionable intestinal tuberculosis, no free space was found in one while a partial collapse was established in the other. Both of these patients are alive but clinically unimproved.

A case with a combined intestinal and laryngeal complication and another with multiple joint tuberculosis were also subjected to artificial pneumothorax. Both of these patients belonged to the extreme hopeless group, upon whom treatment was attempted largely as a matter of psychological expediency. A partial collapse was established in the former case and a successful collapse in the latter. The subject with a partial collapse developed spontaneous on top of the artificial pneumothorax. Both of the cases terminated fatally.

Well-defined laryngeal tuberculosis was present in 9 cases out of our total series of 207. Of these 9 only one sustained a successful collapse and 6 a partial collapse, while in 2 instances the treatment was a failure. Two of the 9 received treatment for the control of haemoptysis. Of the 6 cases with a partial collapse in this group, 2 were greatly improved, one was improved and 3 died. Of the 2 failures, one is being treated at the hospital and the other is dead. The successful collapse case terminated fatally. In our experience only acute tuberculous lesions in the larynx contraindicated pneumothorax treatment.

COMPLICATIONS FOLLOWING COMMENCEMENT OF TREATMENT

Spontaneous pneumothorax: Eight cases of our total series of 207 developed spontaneous on top of the artificial pneumothorax. Of these 8, three had their condition still further complicated by a pyoneumothorax which subsequently resulted in the death of two of the three, while the third is alive but symptomatically unimproved. Of the remaining 5, one is unimproved and 4 are dead.

Pyopneumothorax: Of the total number who have received treatment by induced pneumothorax, 7 developed pyopneumothorax. Three of the 7 have already been discussed in the preceding paragraph as having a spontaneous pneumothorax associated with a pyopneumothorax. Regarding the other 4 cases with this complication, one was greatly improved and 2 died, while the fourth is under observation at the hospital.

Pleural effusion: Pleural effusion is the most frequent complication encountered in pneumothorax treatment. Some investigators have even ventured the opinion that all artificial-pneumothorax cases develop a serous exudate at one time or another, provided the treatment is extended over a sufficiently long period. They also speak of the so called transient effusions, which frequently come and go during the course of the treatment. In our series pleural effusions were demonstrated in only 31 cases, or about 20 per cent. Of these, 58 per cent sustained a successful collapse and 42 per cent a partial collapse. With the exception of 3 cases all were far advanced. The exact cause of the effusion is as yet not thoroughly understood. Our observations, however, lead us to believe that the number of cases that develop serous effusions is in direct ratio to the severity of the lesions in the contralateral lung, and also to the volume and pressure of air introduced into the pleural space. During the first two years of our experience with the treatment, single inflations of air in quantities up to 1500 cc. were not unusual, and, whether as a result of that or not, practically every artificial-pneumothorax case at the National Jewish Hospital at that time had fluid in the chest. Since then a rule has been adopted that in average cases no more than 500 cc. of air, and under no higher pressure than zero, be given for a single dose, and the number of cases that developed serous effusions fell to less than 15 per cent. The 20 per cent of our series mentioned above include all the cases in which large quantities of air, and also under high pressures, were deemed necessary for the control of haemorrhages and for the separation of adhesions. There is another factor which, in our opinion, plays an important part in the causation of fluids, namely, the technique of the administration of the gas. About the time that we commenced to cut down on the dosage of air we also began to use a smaller needle (21 gauge), and we likewise abolished the local anaesthetic except for initial inflations. A smaller needle produces a smaller opening in the parietal pleura and therefore less traumatism and less irritation. As for the anaesthetic, we think that solutions containing novocain or apothesin are bound to act as irritants when they

come in contact with a sensitive pleura. By exerting a little pressure between the ribs at the point of entrance of the needle, we found no difficulty in administering the gas without an anaesthetic.

Gas embolism and pleural shock: Since it is difficult to distinguish clinically, and even postmortem, between cases of gas embolism and so called pleural shock, we classified such cases under one heading. This complication occurred in three cases, with one fatality. In the fatal instance the accident took place at the eighth inflation. The patient stopped breathing and became pulseless immediately after the insertion of the needle, and within about ten minutes he was dead. In another case, during the first treatment, the patient developed symptoms of shock before any air was introduced. He responded, however, promptly to stimulation and regained consciousness in about fifteen minutes. No further attempts to collapse his lungs were made. In the third instance the complication took place during a reinflation after only a few cubic centimetres of air had been introduced. The patient recovered promptly. This, together with the fact that local anaesthesia was used only in a very small number of inflations, would tend to disprove the theory that pleural shock can be avoided by a thorough preliminary anaesthetizing of the pleura. Very little is known about gas embolism and pleural shock, and we know of nothing that is capable of preventing their occurrence.

INFLUENCE OF TREATMENT ON FEVER, SPUTUM AND WEIGHT

Fever: The fact that the efficacy of collapse therapy can often be measured by its influence on the cardinal symptoms of tuberculosis led Fishberg to remark, "When the pneumothorax does not reduce the temperature, we may consider the treatment a failure in this particular case." A perusal of our series of febrile cases revealed that gratifying end-results were obtained among those patients in whom the pneumothorax was responsible for an immediate reduction in temperature, and results somewhat similarly satisfactory were discernible when the treatment, although failing to check the febrile condition of the patient immediately, was successful in subsequent months. Of the 207 cases under discussion in this paper, 100, or approximately 49 per cent, were febrile before treatment. Twenty-eight of the 100 proved inoperable, and further attempts to induce a collapse were abandoned. Of the remaining 72, 15, or approximately 21 per cent, became afebrile im-

mediately after treatment, that is, from two to ten days following the initial inflation. Ten, or about 14 per cent, of the 72 regained normal temperature in subsequent months. In 14 cases the temperature was markedly reduced, while in 7 cases the temperature was reduced to normal but subsequently went up again on account of complications.

In 14 cases of the total number to receive treatment the occasional febrile exacerbations disappeared after treatment.

Of those whose temperature was reduced to normal immediately after treatment, a successful collapse was established in 9, or 60 per cent, and a partial collapse in 6, or 40 per cent.

Of the 9 successfully collapsed cases the present status is designated in 7. Of these 7, four are clinically well, 2 are greatly improved, and one died as a result of a brisk haemoptysis. Of the 6 partially collapsed cases, 3 are clinically well and one is dead. The remaining 2 are under treatment in the hospital wards. With the sole exception of one patient the entire number in this group belong to the far-advanced stage of the disease, with more or less extensive involvement in both lungs.

Of the 10 patients whose temperature became normal in subsequent months, 7 sustained a successful collapse and 3 a partial collapse. Of the successfully collapsed cases in this group, 3 are improved, one is clinically well, one is unimproved, and one is dead. The seventh is under observation in the hospital. The 3 partially collapsed cases are alive and doing well. Nine were far advanced, and one was moderately advanced.

Thirty, or approximately 40 per cent, of the 72 febrile cases with induced pneumothorax, remained febrile throughout their entire hospital residence while 42, or approximately 60 per cent of the 72, had their fever favorably influenced by the treatment.

Altogether, there were 51, or approximately 50 per cent of the entire 100 febrile cases, who retained their fever throughout their stay in the hospital. Only 16 per cent of the 51 sustained a successful collapse, while 43 per cent sustained a partial collapse; in 41 per cent the treatment proved a failure. Of the 49 patients whose temperature was favorably influenced during their hospital residence, 49 per cent sustained a successful collapse, 39 per cent a partial collapse, while only 12 per cent were failures.

Sputum: Of our 154 cases with a definite degree of compression, 140 had positive sputum before treatment, while in 14 the sputum was negative. Thirty-seven, or approximately 26 per cent of the positive

cases, became negative at various periods following the administration of pneumothorax. Thirty, or 84 per cent of the 37, sustained a successful collapse, and 7, or 16 per cent, a partial collapse. Concerning the 30 in this group with a successful collapse, the end-results are designated in 25. Of these, 12, or 48 per cent, are clinically well; 4, or 16 per cent, are greatly improved; and 6, or 24 per cent, are improved. One is unimproved, while 2 are dead; both deaths were due to influenza, and in one instance complicated by pneumonia. Of the 7 partially collapsed cases, one is clinically well, 2 are greatly improved, 2 are improved, and one is dead. The present physical condition of the seventh is unknown.

Of our entire series of 207, 191 had positive sputum, and 42 became negative during their hospital residence. Of the 42 that became negative, 30, or 71 per cent, had a successful collapse, 7, or 17 per cent, a partial collapse, and in 5, or 12 per cent, the treatment proved a failure.

Of the 37 who became negative after treatment, 89 per cent were far advanced and 11 per cent were moderately advanced.

Weight: Of the 207 cases comprising our series the record of the patient's weight change is known in 169. Of these, 70, or approximately 41 per cent, gained weight during their hospital residence, and 76, or approximately 45 per cent, lost weight. In 23 cases, or about 14 per cent, the weight was stationary; that is, although there were at times considerable fluctuations, the weight on discharge was identical in number of pounds with the weight just prior to the administration of treatment. Thirty-one of the 70 who gained weight sustained a successful collapse, 22 a partial collapse, and in 17 cases the treatment proved a failure. Of the 76 patients who lost weight, 30 had a successful collapse, 29 a partial collapse, and in 17 cases the treatment was a failure. It would seem, therefore, that in relation to the two groups of patients,—the lost-in-weight group and the gained-in-weight group,—the ratios of satisfactory collapses, partial collapses and failures were approximately the same.

About 38 per cent of the successfully collapsed cases gained in weight after treatment, and an equal percentage lost weight, while 29 per cent of the partially collapsed cases gained weight and 39 per cent lost weight. Among the failures the percentage of cases who lost and gained weight was the same—32 per cent.

RESULTS ON DISCHARGE

As the maximum time-limit for a patient's residence at the hospital is eighteen months, no higher rating than "greatly improved" is recorded on dismissal. To achieve this rating a patient had to approximate at least a condition akin to being symptomatically well, although there are a few cases included under this heading which, upon discharge, were apparently arrested. Of our 207 cases 46 are still under observation in the hospital wards, and were therefore not included in this group. In addition to the above 46 cases there were 24 patients who had reached the terminal stage of the disease and upon whom pneumothorax was attempted purely as a matter of psychological expediency. The end-results of these cases will be discussed under a separate heading further on. Concerning the remaining 137, 50, or approximately 36 per cent, were discharged greatly improved; 50, or 36 per cent were improved; in 16, or about 12 per cent, the condition remained unchanged; and 7 became worse and 14 died.

Of the 50 patients who were discharged as greatly improved, 64 per cent sustained a successful collapse, and 26 per cent sustained a partial collapse, while only 10 per cent of the 50 were failures.

There were only 26 per cent of the 50 improved cases that sustained a successful collapse, while 44 per cent sustained a partial collapse and 30 per cent were failures.

Of the 16 cases which were discharged unimproved, 38 per cent sustained a successful collapse and 18 per cent a partial collapse, while in 44 per cent the treatment proved a failure.

Of the 7 whose condition became worse on discharge, 14 per cent sustained a successful collapse, 29 per cent a partial collapse and in 57 per cent the treatment failed.

Regarding the 14 fatal cases, 21 per cent sustained a successful collapse, 58 per cent a partial collapse and 21 per cent were failures.

The foregoing data seem to indicate that the largest percentage of failures was among those patients who upon discharge were either unimproved or worse, while the smallest percentage of failures was among those who were discharged as greatly improved. Proportionately, there were approximately twice as many successfully collapsed cases in the greatly improved group as in the unimproved group, and four-and-a-half times as many as in the group of patients discharged as worse. Conversely, there were almost six times as many failures among those

who became worse on discharge as in the greatly improved group, and about twice as many as in the improved group. There were about one-fourth as many failures in the worse-on-discharge group as in the unimproved group.

Of the 50 cases in the greatly improved group, 84 per cent were far advanced and approximately 14 per cent moderately advanced. Of the 50 cases in the improved group, 90 per cent were far advanced and 10 per cent moderately advanced. Approximately 87 per cent of the unimproved group were far advanced and 13 moderately advanced. All of the subjects in the worse-on-discharge and fatal groups were far advanced.

END-RESULTS

The statistical data on end-results are presented here with the full realization that no definite conclusions should be drawn from them as to the value of pneumothorax treatment. There are many factors which play an important part in influencing end-results. Given two cases which are more or less alike as to their lung changes and the character of collapse, the end-results may vary on account of differences of age, sex, occupation, home environment, economical and social status, temperament, intercurrent diseases, and many other conditions which enter into the post-sanatorium life of the patient. It is needless to say that an attempt to classify cases according to the above-mentioned conditions would be an extremely difficult matter.

The present physical status of the patients who have been selected for artificial-pneumothorax treatment during their hospital residence has been determined by means of questionnaires. For various reasons, direct communication with the patient was made impossible in a number of instances, and therefore indirect methods, such as corresponding with distant relatives or with the various charitable organizations which are accustomed to interest themselves in the indigent consumptive, were necessarily resorted to. Although this latter method to obtain authentic information about a pneumothorax case was not entirely productive of desired results, because of the comparative vagueness of the information, nevertheless it conveyed to us sufficient knowledge to classify such cases under the various headings into which our end-results are divided. If our information from the different patients indicated a resumption of normal occupational duties, together with the absence of constitutional symptoms, a classification of "clinically well" was justifiably made.

Aside from the 46 cases which are under observation in the hospital wards, we have tried to determine the present physical condition of the remaining 161. Twenty-four of these belong to the *ultimum refugium* group mentioned below. Of the remaining 137 cases, 35, or approximately 26 per cent, are clinically well; 37, or about 27 per cent, are improved; 9, or about 7 per cent, are unimproved; and 43, or approximately 31 per cent, are dead. In 13 of the total number the present physical condition is unknown.

Of the 35 cases that are clinically well, 57 per cent sustained a successful collapse, 31 per cent a partial collapse, and in 12 per cent the treatment proved a failure. At the time of discharge from the hospital, 71 per cent of the 35 cases received a rating of greatly improved, and 29 per cent unimproved.

Of the 37 that are improved at present, 41 per cent sustained a successful collapse, 24 per cent a partial collapse, and 35 per cent were failures. Of this 37, 46 per cent were discharged as greatly improved, 43 per cent as improved, and 11 per cent unimproved.

Approximately 26 per cent of the 43 fatal cases died from causes other than tuberculosis, in most instances the cause being influenza or pneumonia. This, of course, is a conservative estimate, as a number of questionnaires were simply returned with the information that the patient had died, and no effort was made to impart to us the circumstances surrounding his death. Attempts to obtain such information were usually futile, as the sources with which we had to communicate were, as a rule, unable to enlighten us any further than that the patient had died.

Ultimum refugium group: This group comprises 24 cases of the total number upon which pneumothorax was attempted purely as a palliative procedure. A fatal termination resulted in 83 per cent of the 24. One case improved, while the condition of another remained the same. The present status of two others is unknown.

LENGTH OF TIME ARTIFICIAL PNEUMOTHORAX SHOULD BE CONTINUED

The length of time during which the pneumothorax treatment should be kept up is debatable. Some are of the opinion that from twelve to eighteen months is a sufficient period to continue the collapse. Others, however, believe that the collapse should be kept up as long as possible. Necropsies have shown repeatedly that compression of lungs which

had been kept up for several years failed to effect complete cures of the foci. Furthermore, the rather frequent recurrence of haemoptysis following the reëxpansion of previously collapsed lungs tends to prove that it is never safe to discontinue the treatment. We feel that as long as the patient is doing well the collapse should be kept up unless economical reasons or adhesions filling up the pleural space dictate otherwise.

HOW EARLY SHOULD ARTIFICIAL PNEUMOTHORAX BE ADMINISTERED

The analysis of the above data points clearly toward the fact that the best results were obtained in cases with good contralateral lungs, in which a successful collapse was secured, and which were free of serious extrapulmonary complications, especially enteritis and active laryngitis. The percentage of advanced cases in which such ideal conditions can be found is comparatively small (in our series 38 per cent fulfilled one condition only, namely, a free pleural space), while earlier cases should give a much higher percentage. While this treatment is probably not indicated for lesions which show a definite tendency toward retrogression, it is highly beneficial in progressive cases. If an early case with a progressive unilateral lesion is allowed to go on without interference, his chances for developing pleural adhesions, for the spreading of the lesion to the other lung, and for developing complications, are constantly being increased, and only too often is this treatment postponed until the full benefit of it can no longer be expected. The influence of a few months of proper sanatorium regimen should be a sufficient index as to whether we are dealing with a progressive or retrogressive lesion, and if an early case tends definitely toward progression artificial-pneumothorax treatment should be administered without delay.

CONCLUSIONS

A careful and impartial analysis of the above data suggests to us that they are not convincing enough to justify definite conclusions as to the indications and contraindications for artificial-pneumothorax treatment. We have no entirely satisfactory classification of cases at present, and it is doubtful whether we shall ever have one, for from the standpoint of many important details there are no two cases of pulmonary tuberculosis that are alike. The lack of such a classification precludes the possibility of making observations with proper controls.

However, keeping the above limitations in mind, our data tend to prove the following:

1. That the best results were obtained in cases in which a successful collapse was secured, whose contralateral lung was only slightly involved, and which were free of serious complications interfering with the nutrition of the patient, such as enteritis or active laryngitis.

2. That advanced lesions in the "good" lung contraindicate the treatment.

3. As to all other cases, while the balance of evidence seems to be slightly in favor of the treatment, yet, considering especially the frequency of complications resulting from this treatment in such cases, it is probably best not to be too optimistic about it unless the treatment becomes imperative for the purpose of controlling haemoptysis. A reasonable trial should be given first, to other forms of immobilization, such as strict bed-rest, posture treatment or the chest-belt.

4. That small quantities of gas under low pressures tend to minimize the occurrence of pleural effusions.

5. That local anaesthesia is not necessary and is probably harmful.

6. That the treatment should be kept up as long as possible.

7. That the treatment should be administered to all early cases whose lesions show a definite tendency toward progression.

ACCIDENTAL PNEUMOTHORAX COMPLICATING AN ATTEMPT TO INDUCE ARTIFICIAL PNEUMOTHORAX[1]

SALING SIMON

Among the immediate accidents that occur as a consequence of the institution of artificial pneumothorax the most serious one is probably that of air embolism or the so called pleural shock of the earlier literature from 1864 to 1900. The pleural eclampsia or pleural epilepsy of Roger, Besnier, Legroux, Lépine, and others, occurred during irrigation of a chronic empyema cavity, during diagnostic puncture of the chest-wall, during the injection of bismuth paste for diagnostic purposes, and finally in more recent years during the institution of artificial pneumothorax (1). However, the convincing experiments by Brauer's pupil, Wever (2), trace practically all fatalities following exploratory puncture of the chest to gas embolism, and leave only the possibility of minor nonfatal disturbances attributable to pleural reflex. In a large experience with artificial pneumothorax practically every tuberculosis specialist meets an occasional accident, such as air embolism (3), giving rise to striking and alarming symptoms, which make him alert for the unusual, and ever watchful to obviate disasters to his patient. He frequently sees conditions develop in the patient which no doubt are not such rare accidents and have in the past to a great extent been overlooked or not recorded. The literature on air embolism following various diagnostic or therapeutic procedures in diseases of the pleura and lungs contains numerous references reviewed in a former article (3), according to which cases have developed grave symptoms attributable to air embolism immediately following the introduction of the needle, and there are quite a few cases on record in which spontaneous pneumothorax occurred at various intervals after the institution of artificial pneumothorax (4) (5) (6), but cases in which an accidental pneumothorax developed immediately upon the introduction of the needle used for instituting primary artificial pneumothorax are exceptional.

[1] From the National Jewish Hospital for Consumptives, Denver, Colorado.

Wallgren (7), in reporting on spontaneous pneumothorax as a complication of artificial pneumothorax, records three cases in which spontaneous pneumothorax occurred shortly (2 to 8 hours) after the institution of artificial pneumothorax. As a result of his studies, he believed that this was due to lung perforation which may occur: (1) During the first insufflation, as a result of needle injury to the lung, when symptoms may appear hours afterward and the prognosis be favorable. (2) When an adhesion occasions a tear in the lung and the prognosis may be favorable. (3) When a surface tuberculous focus, in progressing, perforates during lung compression, and here the prognosis is bad. (4) When a weakened healing lung area ruptures as a result of diminished pleural pressure or trauma and the prognosis is unfavorable. (5) When pyogenic empyema complicates artificial pneumothorax, ulcerates through into the lung and thus discharges, and the prognosis is bad.

Giesemann (8) in reporting on the accidents and complications of artificial pneumothorax in 1918, records three cases in which accidental pneumothorax occurred following the introduction of the needle only. In one the needle was inserted once, and immediately symptoms developed, with pain at the site of the needle insertion, in the right shoulder and right arm, and fifteen minutes later the patient was pale and the pulse slow, but otherwise the course was uneventful. Pneumothorax was found upon X-ray examination; it persisted three days, and on the fourth day more air was injected with a favorable outcome. In the second case the needle was also inserted only once, when the pneumothorax developed, and four days later 400 cc. of oxygen was introduced into the space still persisting, and lung collapse was maintained by repeated injections at spaced intervals. The third case was that of a very nervous and irritable patient, in whom a dull needle was inserted in three different places without manometric oscillations, after which the patient was very restless and became pale. One hour later he complained of a "stitch" in his side and the feeling of "gas leaving the lung and entering the pleura." Examination one hour after this revealed a large basal pneumothorax. Three days later gas was introduced to maintain collapse, and the pneumothorax was found to be of the closed variety. No exudate formed in this case. Giesemann also reported a case in which there occurred a small valvular perforation of the lung, the reverse of the usual type, allowing a successful artificial pneumothorax to empty itself by way of the lung.

There was found in the literature just one other case of this type,

accidental pneumothorax, described by Sedlmeyer (9) in 1921. An attempt was made to institute an artificial pneumothorax in a 32-year-old patient, but no manometric readings were obtained on inserting the dull needle in four different locations in the chest. No gas was introduced. Shortly afterward the patient, who had been quiet previously, suffered a severe coughing spell, during which there occurred a stabbing pain in the chest followed by shortness of breath. Inhalation of oxygen afforded relief. The pulse was increased but regular, and there was no cyanosis. During the next hour there developed a widespread emphysema of the chest. An X-ray, taken eight days after the accident and when the skin emphysema had disappeared, showed a pneumothorax of the left side, which was then maintained by repeated refills of gas. The subsequent course of the case was uneventful.

The case to be reported below is presented because there appears to be no similar case in the American or English medical literature, and for its value in adding to the already existing reports of accidents occurring during the administration of artificial pneumothorax another of unique origin.

The patient, C. F. G., was 38 years old, married, and had two children. He was a conductor by occupation. His mother died of tuberculosis at forty years; otherwise the family history was negative. During childhood the patient had had measles, whooping cough and smallpox. He had had no other important diseases until the onset of the present illness, which dated from September, 1923, at which time he complained of pains between the shoulders, night sweats, and slightly blood-tinged sputum. He gave no history of cough or fever. The diagnosis made at the time was pulmonary tuberculosis, and the patient was treated in St. Louis for about one year without much improvement. He came to Denver in October, 1924, and was first seen and examined by the author in February, 1925, when he presented the following complaints: Progressive weakness, aching pain between the shoulder blades, and aching pain in the pit of the stomach associated with acid belching which was not influenced by eating or diet. He had a slight cough, with a small amount of expectoration which was blood-tinged at times, as well as slight dyspnoea and night sweats. Tubercle bacilli had been found on two separate examinations.

Physical examination at this time disclosed some dulness at the right apex, with a few fine râles. The left lung was extensively involved throughout, and there were numerous large moist râles. The patient failing to improve under

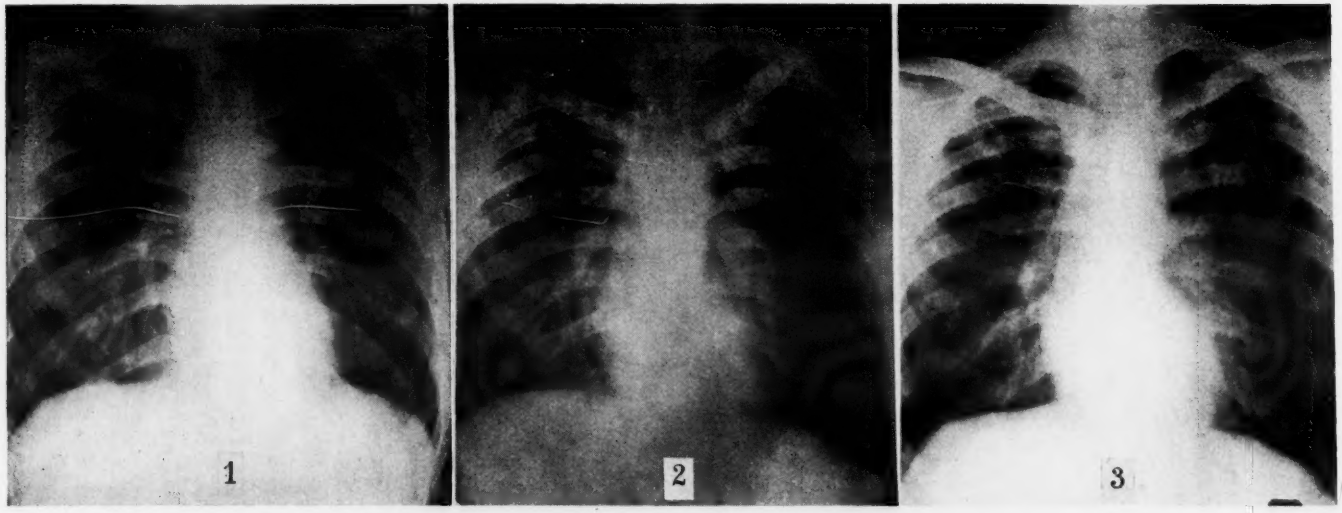

FIG. 1. ROENTGENOGRAPH TAKEN BEFORE THE FIRST ACCIDENTAL PNEUMOTHORAX

FIG. 2. ROENTGENOGRAPH TAKEN ONE DAY AFTER THE FIRST ACCIDENTAL PNEUMOTHORAX AND SHOWING THE GAS IN THE LEFT CHEST WITH COLLAPSE OF THE LEFT LUNG, AND MEDIASTINAL AND CARDIAC DISPLACEMENT

FIG. 3. ROENTGENOGRAPH TAKEN SHORTLY AFTER THE SECOND ACCIDENTAL PNEUMOTHORAX AND ON THE SAME DAY

ordinary treatment, he was advised to undergo artificial pneumothorax treatment.

On March twenty-third an area between the fifth and sixth ribs in the mid-axillary line was anesthetized with 0.5 per cent novocain solution containing 1:10,000 adrenalin; and the needle, 20 gauge, and 2 inches in length and connected with the manometer of the pneumothorax apparatus, was inserted in this area (Forlanini puncture method). There was no apparent difficulty in entering the pleural space, and a reading of -2, -4 was obtained. The cock leading to the manometer was turned off, and just as the cock connected with the air bottle was to be turned on the patient complained of distress, shortness of breath and palpitation of the heart. No air had been allowed to leave the pneumothorax apparatus, and, despite this fact, when the manometer cock was again turned on a reading of plus 2 was obtained. The pneumothorax needle was withdrawn and the patient given a hypodermic injection of atropin. One hour later fluoroscopy was possible and the left lung was found collapsed. The next day an X-ray picture of the chest was taken and showed a marked collapse of the lung by air. The patient complained more or less of pain in the left side of the chest, of a "catching nature," especially when attempting to take a moderately deep breath. About two weeks after the accident the patient still complained of slight pain, and an X-ray picture of his chest at this time showed an almost complete absorption of the pneumothorax air.

On April twentieth another attempt to induce artificial pneumothorax was made, when the technique used in the previous trial was repeated. The pleural space was again readily entered, when the patient complained of exactly the same symptoms as those occurring on the first occasion. It was deemed advisable to discontinue the attempt, and the needle was quickly withdrawn. X-ray of his chest on the same day again showed a collapse of the left lung. The patient refused further pneumothorax treatment after this. In May another X-ray picture of his chest disclosed that the air had completely disappeared from the pleural space.

While the patient complained a great deal of difficulty in breathing, and of distress in the left side of the chest and over the cardiac area, following the two accidental pneumothoraces, at no time did his condition appear alarming or serious, for while the pulse rate was increased it maintained a fairly good quality. The patient acted in every way like one who had been given an excessive amount of gas into the pleural space.

DISCUSSION AND SUMMARY

Numerous reports are found in the literature in which an accidental (spontaneous) pneumothorax followed an attempt at induction of arti-

ficial pneumothorax but no direct relationship between the two was traceable, except in the few cases cited by Giesemann (1918) and Sedlmeyer (1921), because the interval between the discovery of the accidental pneumothorax and the attempted induced pneumothorax was from eight to twenty-four hours, and in some cases extended to several days, while in many cases air was actually introduced.

When air embolism or accidental pneumothorax occur upon the first introduction of the needle into the chest for artificial pneumothorax, and before the actual introduction of gas, the explanation would seem simple, and yet one is left to wonder why these accidents are then not more frequent during the course of pleural tappings, exploratory punctures and, above all, the initial inflations in artificial pneumothorax. Forlanini early maintained, as a result of animal (dog) experiments, that the careful introduction of a pointed needle into the pleura resulted in no injury to the lungs or blood-vessels; while Brauer, in direct opposition, found it difficult to avoid such injury, even with a blunt needle, in dogs, and for this reason exposed the pleura (by incision) before introducing the needle for instituting artificial pneumothorax.[2] Even if the truth lay between these two conceptions, it is difficult to explain the rarity of the accidental pneumothorax during the usual therapeutic or diagnostic chest-puncture procedures, unless it can be attributed to some abnormality of the lungs of the patient under consideration, similar to that believed by Forlanini to exist. The latter maintained that puncture of the lungs during the institution of artificial pneumothorax is without harm when the punctured lung is free from tuberculous changes, but if an emphysematous condition of the punctured area exists penetration of the emphysematous bleb can occasion a spontaneous (accidental) pneumothorax.

SUMMARY

There is reported a case of accidental pneumothorax complicating pulmonary tuberculosis, in which the needle for artificial pneumothorax was introduced into the pleura, manometric oscillations were obtained and, before gas could be introduced from the pneumothorax apparatus, the symptoms of pneumothorax developed and the condition was verified by fluoroscopic and roentgenographic examination, and the occurrence

[2] Dr. H. J. Corper informs me that it is extremely difficult to avoid injury to the lung in introducing a needle into the pleural space of normal dogs and rabbits for the purpose of making intrapleural injections.

was repeated about one month later, and after the pleural air from the previous accident had completely absorbed. Since there seems to be no other explanation to account for the entrance of the air into the pleural space, except through the needle-injury area in the lung, it would appear that the lesion assumed a valvular form which, with collapse of the lung, completely closed, and healing prevented its further functioning in this capacity, very much like a valve produced in rubber tubing by means of a longitudinal straight incision, self-sealing in collapse and open during distention. The repetition of the accident may be attributed to the existence of a pulmonary abnormality (emphysematous blebs) mentioned by Forlanini.

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METALLIC SOUND

HUGH M. KINGHORN

If there are present within the thorax large, somewhat rounded cavities of at least 4 cm. (about 1½ inches) longitudinal diameter, which conduct air, or air and fluid, and possess smooth walls, sound phenomena which develop in them or which are conducted from their vicinity can be associated with metallic sound at this place. By metallic sound is meant that same high and clear sound which accompanies the bursting of the larger bubbles of spittle in the rounded opened cavity of the mouth, which is heard when fluid is shaken in a jug, or which resounds when one knocks on empty barrels.

Metallic sound, like the metallic sound arising in percussion, presupposes that there are cavities which permit the reflection of sound waves from all directions, that is, it presupposes the formation of a closed-in wave system.

The pitch of the metallic sound depends essentially on the largest diameter of the air-space in which it arises. By pitch I mean the place which the musical tone occupies in the musical scale. We can determine the length of this diameter by comparing artificially constructed air-spaces of a similar pitch of sound. It is not correct, however, to assume that the width of the opening of such a space has no influence on the pitch of its metallic sound. We can prove this first, by holding together wide chest fistulae which lead into a pneumothorax, and secondly, if we percuss a glass bottle and at the same time slowly close its mouth and simultaneously auscultate with the ear. Biermer also found that the tension of the wall has some influence on the pitch of the metallic sound.

Not always, but in the great majority of cases in which the percussion sound has a metallic sound, metallic sound is also heard in auscultation. It can also be induced by breathing, by the whispered and spoken voice, by râles, and sometimes by whistling. By this I mean that percussion sound, breath-sounds, vocal resonance, râles, and sometimes whistling, may have metallic sound. It is immaterial as to whether the cavity which produces the metallic sound is connected with the corresponding bronchi or not, also whether these inciting sounds arise in the cavity itself or in the vicinity.

Metallic sound is most frequently produced by râles. Gerhardt (*Lehrbuch der Auskultation und Perkussion*, p. 196) found that it generally happens that among a large number of nonmetallic sounding râles he could distinguish only here and there a clear high-pitched metallic sound. This fact is sometimes well demonstrated by listening to the râles produced by cough over an artificial pneumothorax. Here we will sometimes obtain a shower of râles, and will perhaps hear only one râle with well-marked metallic sound. When we hear metallic sound accompany râles it is also called metallic tinkle. To confirm the fact that metallic sound is more frequently and more distinctly prduced by râles, we can listen over an artificial pneumothorax. Here I have been sometimes unable to get metallic sound by normal breathing or the spoken voice; by whisper it was distinctly present, but, on coughing, metallic-sounding râles were distinctly heard.

Metallic tinkle is a sign of much value in pneumothorax, and its mode of production has been the subject of much speculation and discussion. It is a clear vibrant musical sound, and is heard in many cases of hydropneumothorax. It has been likened to the distant tinkling of a clear, highpitched silver bell. According to Walter B. James (Footnote, *Osler's Modern Medicine*, 1907 ed., iii, 881) the sound is characteristic, and resembles nothing else that is heard over the chest. It may occur during normal respiration, or be produced only upon deep inspiration or by coughing. Sometimes it will occur after change of position. It is heard only over the affected portion of the chest, and is generally associated with, but need not necessarily be accompanied by amphoric breathing. The sound is best described by the terms *metallic* and *tinkling*. The following explanations have been given to account for its formation: (1) that it is produced by drops falling upon fluid from the inner surface of the chest; (2) by the bursting of bubbles on the surface of fluids; (3) by air bubbling up through fluid from a submerged fistula.

Norris and Landis (Footnote, *Diseases of the Chest and the Principles of Physical Diagnosis*, 2nd ed., p. 131) illustrate the causation of metallic tinkle by the following experiment: If we attach a stethoscopic tubing to to the mouthpiece of a wash bottle and listen while air is being forced through the other tube into the liquid, the phenomenon of metallic tinkle can be accurately reproduced. The sound also occurs at the moment the bubble is given off and not at the time it reaches the surface. If, however, we listen while water is allowed to fall from a pipette upon the surface of the water, only a dull indifferent sound will be heard.

These authors therefore conclude that metallic tinkle is due to air bubbles ascending through an effusion from a fistulous tract in the lung below the level of the liquid, and not to liquid dropping from a moist pleura upon the effusion beneath. It seems to me that this explanation is probably not correct, for when we produce an artificial pneumothorax by air or nitrogen gas we frequently obtain a well-marked metallic tinkle. We know that there is no fistulous opening in the lung, and that no air passes from the lung to the pneumothorax space.

These authors also state that metallic tinkle may also be produced by the bursting of bubbles in a bronchial tube which communicates with a pneumothorax, or by a bubble in the surface of a moist perforated lung above the level of the fluid, if the bubble is impelled with sufficient force. In either case the musical quality is due to the rhythmic vibration of the air, and the reverberation is due to the large air-filled, stiff-walled resonator,—the pleural cavity.

Walter B. James regards the most reasonable explanation to be, in view of all the facts reported, that metallic tinkle is merely a râle, or what, under other conditions, would be a râle, but which is modified through being produced in or near to a resonance chamber which, acting as a sounding-board, gives to it its peculiar metallic quality. In a few cases the patients themselves have been able to hear the metallic tinkle, and in a case reported by Allbutt it could be heard in all parts of a large room.

In rare cases metallic tinkle may be heard over tuberculous cavities in the lung when the physical conditions approximate those present in pneumothorax. In several of my cases of almost complete excavation of the lung it was very distant.

Gerhardt is of the same opinion with regard to the production of metallic tinkle as is James, and believes that is it the râles which produce the sound. It is generally a striking fact that among a large number of non-metallic sounding râles, one distinguishes only here and there a clear, high-pitched metallic tinkle. It was assumed that the latter was temporarily induced by drops falling within the cavity, but one cannot imagine that merely drops should induce that which râles, which are present, do not induce.

We can change loud ringing râles artificially into metallic tinkle by placing a suitable resonator on the corresponding place of the chest-wall. We can become convinced that it is strong resonance of a high-pitched overtone which is the cause of this peculiar phenomenon. Gerhardt assumes that it is one or more overtones which are much higher pitched

than the basic tone, and which accompany the latter independently and then vanish slowly, that give the ear the impression of metallic tinkle. This explains the rare occurrence of this strong clear sound, the pitch of which is, under ordinary conditions, always the same.

Metallic sound is heard still more rarely with tubular breathing and bronchophony than with râles. From this explanation we will obtain something of an idea of the space of the cavity by determining the pitch of the metallic sound in the musical scale.

The falling drop as the cause of metallic tinkle was particularly brought into prominence by von Leichtenstern (Gerhardt's *Lehrbuch*, p. 196, footnote), who demonstrated that in a large cavity—for instance, in a pneumothorax cavity—the metallic sounds of the falling drop can still be induced in a corpse, and at the place where it was heard in the living body. This, however, is not the rule.

Over cavities which produce metallic sound we hear breath-sounds sometimes accompanied by deep, hollow buzzing. These sounds are similar to those produced by blowing into a hollow jug. This sound is called amphoric echo, and possesses the same significance as metallic sound. It represents a form of metallic sound with a very low basic tone. Amphoric echo can be produced not only by breathing, but by ordinary speech and by whisper. The vocal resonance has then an amphoric quality. Such an amphoric echo is often more distinct and characteristic with whisper than with ordinary speech. Amphoric echo is low-pitched and of "buzzing" rather than "ringing" quality. The metallic quality of the sound is present in amphoric echo, but is less marked. Metallic breathing is found only over very large cavities with smooth walls and of regular shape. Amphoric echo and metallic sound—of like value in their importance,—are often called by one name, *metallic phenomena*. They arise over very large cavities or pneumothorax cavities. They are the most certain symptoms of the presence of the one or the other condition, and yet they do not indicate these with absolute certainty.

Metallic sound was heard by Kolisko and Wintrich (Gerhardt, p. 197) over cavities as large as pigeon's eggs, which communicated with wide branches of the air-passage. These wide branches of the air-passage seem to have enlarged the space of the cavity to such an extent that both together could produce metallic sound.

Friedreich (Gerhardt, p. 197) emphasized the fact that in very rare cases amphoric breathing is heard in the scapular region in healthy lungs.

Skoda (Gerhardt, p. 197) mentions that this form of breathing can be induced in the mouth-cavity in extreme dyspnoea, and that it can be observed over the whole thorax. Gerhardt heard diffuse metallic-sounding râles in a case of extensive cylindrical dilatation of the bronchi of a lung with obliteration of the interstitial tissue, without there being anywhere any large cavity (p. 197).

Our knowledge with regard to metallic sound has been greatly increased by Biermer's (Gerhardt, p. 197) very important demonstration that with the change of the longest diameter of the cavity its pitch changes. When easily movable fluid is present in the cavity in sufficient amount metallic sound will appear higher or lower pitched in a sitting posture than in a lying-down posture (*Biermer's change of sound*). This observation was confirmed by Geigel and others. I have observed this phenomenon in cases undergoing artificial pneumothorax when fluid has developed. A distinct change in the pitch of the metallic sound can sometimes be noticed when the patient changes from the sitting to the reclining position. In one case the pitch was lower with the patient sitting than with him reclining. Biermer's change of sound has since been generally acknowledged, and is an important symptom of the pneumothorax picture. Gerhardt tried to obtain this symptom on a small, sacculated pneumothorax, but failed. With large free pneumothoraces, however, he was easily able to confirm it.

From later communications by Biermer concerning this subject, metallic sound with pneumothorax becomes lower pitched while the patient is sitting than was previously found while the patient was lying down; in one case to the extent of a minor third, in another case to the extent of a fourth of a musical note. According to this, the air-space becomes longer in a sitting-up position, whilst *a priori* we should expect the opposite. From this we must assume that the position of the diaphragm becomes deeper. When the pull of the lung ceases the diaphragm lacks firm tension and becomes relaxed. With the patient lying down the abdominal organs press the diaphragm upward; during sitting or standing they draw it downward. There is also, in addition, the weight of the fluid which is poured out on the diaphragm in the upright posture. With healthy persons the diaphragm sinks to some extent while standing; when pneumothorax is present it sinks quite considerably. Cases of opposite relations also occur, and Biermer reported one such case. Sometimes metallic sound becomes higher pitched in the sitting position, and lower pitched in lying down. Gerhardt states that there is never a

completely free pneumothorax, inasmuch as adhesions connect diaphragmatic pleura and costal and pulmonary pleurae, and limit the motility of the diaphragm. In this event the fluid which is deposited over the diaphragm during sitting shortens the longest diameter of the air-space. Biermer also showed that metallic sound is heard higher pitched over pneumothorax during inhalation than during exhalation.

In the abdomen, metallic-sounding râles are found in the dilated stomach and large intestine; also in cysts which contain air; also in the peritoneal sac when it is full of air, and is produced partly through pressure or shaking. Metallic-sounding, aortic sound also occurs in the last mentioned condition.

It is also well to remember that metallic sound may also, in rare cases, be heard over tuberculous cavities in the lung when the physical conditions approximate those present in pneumothorax. I recall two cases of tuberculous excavation of the lung in which practically the whole lung was excavated. Metallic sound and other signs of pneumothorax were present, but there was no fluid and no succussion splash and no transposition of organs. The diagnosis between cavity and spontaneous pneumothorax was in doubt for some time.

SUCCUSSION SOUND

There are other names for this phenomenon, such as *splashing sound, succussion splash, shaking sound, Hippocratic succussion* (*succusio Hippocratis*). Here also metallic sound is present, but is produced in another way. Fluid which is contained in many large hollow spaces can be set in motion by shaking, and induces a splashing sound, accompanied by metallic sound. This sound is similar to that which we produce when we shake a small amount of water in a jug. Succussion sound is sometimes so loud that it can be heard over the entire sick-room. At other times it is so faint that it can be heard only with the ear pressed to the chest-wall. Its pitch is always the same as the metallic sound associated with it. The popular assumption that succussion sound is heard only in pneumothorax is completely erroneous. Although large cavities are frequently found in the lung, these rarely cause succussion sound. Nevertheless, positive cases of succussion sound in lung cavities have been described by Gendrin, Weber and others (Gerhardt, p. 199). This sound rarely occurs in tuberculous cavities. It occurs far more frequently in lung gangrene or in abscess formation.

The contents of these cavities which develop as a result of lung abscess or lung gangrene usually consist of thin fluid. Gerhardt was able to hear a splashing sound on the chest-wall of bronchiectatics when he shook them. He found, however, that this sound usually lacked a metallic quality and therefore sounded differently. He therefore differentiated it from that of pneumothorax as *nonringing metallic sound*. Metallic sound can be induced by heart action both in adjacent pneumothorax spaces and also in the pericardium when it contains air. This sound is generally produced by râles which occur synchronously with cardiac contraction.

The view is popularly held that metallic-sounding succussion splash should be considered the most important symptom of pneumothorax. Gerhardt, however, relates the following case (page 199):

A man about 60 years of age had suffered for several weeks with lung gangrene. Pleuritic rubbing had been heard for several day. Overnight the rubbing disappeared, and there was profuse expectoration. All the symptoms of pneumothorax appeared, especially strong metallic-sounding splash, but voice buzzing remained strong and the side did not widen out. Rib resection was performed, followed by incision of the area. There was adhesion of pleural surfaces, and a large gangrenous cavity in the lung.

Metallic-sounding succussion sound may also occur in the stomach. It can often be easily differentiated by the character of the tone and the direction from which it comes. It is similarly so with cardiac systolic metallic-ringing râle sounds, which occasionally arise in the stomach.

Succussion splash is a positive indication of the presence of both gas and fluid in a cavity. It is one of the most valuable signs of pneumothorax, but, as shown by Gerhardt's case, not an absolutely positive sign. When we obtain it we must determine that it is made in the pleura and not in some other body cavity. It can be readily distinguished from the succussion sound heard in pneumopericardium, as in this the phenomenon is synchronous with the heart-beat, and produces a peculiar churning sound. It is to be differentiated from splashing sound produced in the stomach or colon, by a careful study of its location and by associated symptoms and physical signs. As mentioned above, it is occasionally heard over a tuberculous cavity of large size containing considerable fluid when physical conditions closely resemble hydropneumothorax, and sometimes the differential diagnosis between these two conditions is difficult.

The first mention of succussion splash is generally ascribed to Hippocrates, hence the term *Hippocratic succussion*. Laennec, however, was

the first to clearly recognize its meaning. He gave a comprehensive description of its mode of production. Succussion sound differs much in intensity in different cases, and these differences are not easy to explain. Only the coexistence of gas and fluid can be concluded from its presence, and not the quantity of either. As a rule, succussion sound is heard without difficulty. Sometimes it is heard best when lying down. In other cases it can be detected only when the patient stands or sits erect.

Succussion splash is one of the most valuable indications of pneumothorax, and it is often obtained before the presence of fluid can be made out by percussion. In tuberculous lung cases undergoing artificial-pneumothorax treatment there is occasionally a sudden attack of fever. These patients should then always be tested for succussion splash, as it is an early and positive sign of fluid. I have never experienced any ill effects to the patient by shaking him vigorously enough to elicit the sign. When we are in doubt as to the exact nature of a condition in the pleura, it is a good rule always to listen for succussion splash. It never occurs in simple serous or purulent effusion, as no air is present. It and metallic tinkle are the most pathognomonic signs of hydropneumothorax.

COIN SOUND

Other terms for coin sound are *bell sound* and *Bruit d'Arain*. Trousseau is said to have first described it, and he made use of an ordinary pleximeter and percussion hammer. As is well-known, to produce the sound one uses two large coins, and applies one firmly to the chest wall, and strikes it sharply with the edge of the other. This can be done by the patient or by an assistant. The listener applies his ear or stethoscope to the chest-wall, and hears a sound which possesses a clear, musical, bell-like quality, which is perfectly distinctive, and the timbre of which strongly suggests that of amphoric breathing.

It is not certain whether the sound is produced by high or low tension of the contained gas. James (p. 882, footnote, *loc. cit.*) thinks that it is most likely produced by the same conditions that give rise to amphoric breathing. It is a very common phenomenon in pneumothorax, and is of great value in diagnosis in certain cases. It may be absent when the pneumothorax is less extensive. It is, however, not pathognomonic of pneumothorax, as I have obtained well-marked coin sound in two cases of almost complete tuberculous excavation of a lung.

EDITORIAL

TUBERCULOSIS THE FIFTH CAUSE OF DEATH IN THE UNITED STATES

The continued decline of tuberculosis mortality throughout the United States has been a first-class fact of epidemiology for a decade; but the magnitude of the event, and especially the revolutionary shifting of the elements that make up general mortality, must astound even the most enthusiastic antituberculosis "shouter" of 1915. "No tuberculosis by 1915" was only one more shibboleth born of a wish as father to the thought, and grounded neither on good science nor on sound experience; but the time has come when the annual casting of the figures bids us ask whether it is rash or foolish to set the date of conquest at, say, 1950.

The National Tuberculosis Association, with its old cumbrous though more purposeful name, was launched in 1904, a year of 201 deaths per 100,000 population from tuberculosis in the Registration Area of the United States. Thirteen years later saw the founding of the AMERICAN REVIEW OF TUBERCULOSIS; in 1917, with its 147 tuberculosis deaths per 100,000 population. In 1904 tuberculosis was entrenched where it had always been, at the peak position of human affections. By 1917 heart disease was challenging and passing it, not so much because (at the time) deaths from diseases of the heart were mounting as because tuberculosis was declining.

There has just come to hand the issue of the *U. S. Public Health Reports*,[1] which tabulates the principal causes of death for 1924. In this year 1,173,990 people died within the Registration Area, which comprises 88.4 per cent of the estimated population of the country. The total death-rate was 11.9 per 1,000 population; or a little lower than in 1923 (12.3 per 1,000) and a little higher than in 1922 and 1921 (11.8 and 11.6 per 1,000 respectively).

The deaths from tuberculosis, all forms, numbered 89,724. Therefore, only one of every 13+ deaths was due to tuberculosis. Consider what an advance this ratio implies, as we recall that a century ago the toll of tuberculosis was one out of every three or four deaths, a generation ago

[1] Principal causes of death, 1924, *U. S. Public Health Reports*, January 1, 1926, xl, no. 1, 12.

one-seventh of all deaths, and but a decade ago (so we told our audiences) one-ninth or one-tenth of all deaths. Meanwhile, let us remember that throughout these periods the total death-rate has fallen enormously.

In 1924 the tuberculosis death-rate was 90.6. It had, then, during the first twenty years of the National Tuberculosis Association, declined—"shot down" might be the more apt term—from 201, or 55 per cent. In the thirteen years between 1904 and 1917 the diminution was 27 per cent (from 201 to 147); in the seven years from 1917 to 1924 it was 38 per cent (from 147 to 91). The accelerating momentum of more recent years is more eloquent of what is going on in tuberculosis than pages of commentary would be.

Noteworthy above all is the change in position of tuberculosis among the leading causes of death. From the *Public Health Report* mentioned we gather the following data concerning the first six major diseases:

	NUMBER OF DEATHS IN 1924	RATE IN 1924	PLACE AS CAUSE OF DEATH IN 1924	RATE IN 1923	PLACE AS CAUSE OF DEATH IN 1923
Diseases of the heart	176,671	178.4	1	175.3	1
Pneumonia (all forms)	97,403	98.4	2	109.0	2
Cerebral hemorrhage and softening	91,941	92.8	3	90.4	4
Cancer and other malignant tumors	91,138	92.0	4	89.4	6
Tuberculosis, all forms	89,724	90.6	5	93.6	3
Nephritis	88,863	89.7	6	90.1	5

When, about 1915, heart disease forged ahead of tuberculosis, we regarded it as a phenomenon of the first importance; for, although tuberculosis mortality had been diminishing for years, it had nevertheless clung to the top, and when at last another disease did pass it we were heartened by a concrete and visible symbol of the fruit of our labor. Here finally was something definite, something that conveyed a meaning to even the inexpert.

For a few years heart disease and tuberculosis ran nip and tuck, although the former kept its precarious position. But since 1920 the gap between the two diseases has widened enormously, as heart disease has gone ahead by leaps and bounds while tuberculosis has been retreating with almost equal velocity.

Into the gap, meanwhile, has come pneumonia, which will likely keep ahead of tuberculosis as long as the latter maintains a rate well under 100. However, pneumonia is a disease, or a category of diseases, subject to more violent annual variations than either heart disease or tuberculosis.

Being infectious, it is also theoretically preventable, and amenable to therapeutic and prophylactic measures, once these are found. Its position may, therefore, change radically at any time.

But the year 1924 saw two other diseases for the first time outstrip tuberculosis, which, by their very nature, are likely to keep ahead of it. All signs point to a continued increase of cerebral hemorrhage and cancer, and there is nothing to indicate that they will fall behind tuberculosis for a long time to come.

Tuberculosis in fifth place of killing diseases embodies really a new concept. It is not so easy to get as excited as of old over a disease well down on the list. We must surely find it more difficult to enlist sympathy and active aid in the combat against what is approaching the foot of the scale of major ailments. When we take nephritis into our family we have, according to present classifications, about exhausted the more malignant maladies of mankind. And with nephritis responsible for less than a thousand fewer deaths than tuberculosis in 1924, and tuberculosis continuing to decline, it is quite likely that at this writing tuberculosis has retreated still further, to the sixth cause of death.

Yet, if there ever was a time that called for the putting forth of every effort against tuberculosis it is now. When an enemy, perhaps demoralized, is in retreat, good strategy always counsels that forces be redoubled in pursuit, and permits no slackening until the foe be overtaken and made captive. The Great War taught all intelligent men a lesson in tuberculosis that intelligent men will neither forget nor disregard—the demonstration that as long as tuberculosis smoulders among a people its embers can quickly be fanned into a conflagration,—and the corollary that the fires of tuberculosis are never under control until they are extinguished. In July, 1914, of all great nations Germany could point to its record as the best in tuberculosis; by July, 1918, it was aghast at a "White Plague" that had more than doubled and taken on the dimensions of a century before. As long as we, or any other corner of the world, have *any* tuberculosis, we tolerate a menace. We harbor a threat to national efficiency that is likely to be made good at the very time when our welfare requires efficiency at the peak.

With tuberculosis in fifth, and perhaps in sixth place, we may surely plan more confidently the conquest of tuberculosis. We may go ahead with full knowledge that "time and tide" are with us; indeed, it is with and through "time and tide" that we are working and accomplishing our object. We know that of the miracles of the still adolescent twentieth

century the unexampled increase of wealth and the harnessing of electricity, for man's easement, have been the two really significant historical events in the United States, and that from these have been born almost all else that will go down in our annals for the period. To get, and to hold what we have, and to expend this upon a new power for our comfort and the further increment of new wealth, have put us in Asia and the Caribbean, have cut through Panama, consolidated our industries, yoked man to the machine, and pushed us into the European cauldron. Whatever the Jeremiahs of the "downtrodden masses" may cry out concerning a widening proletariat and the latter's deepening economic abyss, the fact remains that there are twenty million motor-cars in the United States, most of them for "pleasure,"—the recreation of only five times that many inhabitants,—that silk and fur are the ordinary covering of those whom we yesterday called poor, and that into by far the greater number of "humble" homes go the incandescent light, central heating and the bath-room. Hand in hand, hours and opportunities for leisure have lengthened out.

Of course, all this has made for less tuberculosis. No sensible being has ever denied that, as the mass of mankind becomes more cleanly and more comfortable, more protected against cold and heat, more uplifted psychically through the satisfaction and content created by more and better clothing and by increasingly livable homes and neighborhoods, more master of its time,—that mankind then strengthens its armor against the creature of filth and the garret, of darkness and the rookery, of raggedness and exposure, of surfeit of labor and grinding toil, of indigence and despair;—for such tuberculosis surely is, and, in addition, the step-child of the more immediate grisly troop that follow in want's train—the residuary legatee of a host of maladies engendered by dirt, density and deprivation.

But it was organized effort that realized what made tuberculosis and what lay at hand—what was going on—to unmake it. It was the National Tuberculosis Association and its hundred and more subsidiary allies that seized upon the trend of events and turned these to their purposes. Surely the hand of the National Tuberculosis Association is to be seen in the domestic, school and industrial architecture of our time. Much have its calls to the out-of-doors—its behests for the open window, for recreation in the sun and air, for leisure more intelligently employed—guided, yes changed, the habits of the people. Greatly has it cleansed the atmosphere breathed by the miner, the quarryman, the knife-grinder,

and a host of kindred artisans. With good effect has it counselled without ceasing the use of the country's wealth in every way that ameliorates existence. All this to one main purpose—to lessen tuberculosis,—as meanwhile it brought forward proof after proof that current social and economic tendencies could be embodied into more concrete measures against tuberculosis. And this is but a hint of the work of the Association in its more "secular" aspects. At the very least, the organized "campaign" crystallized and defined the object to be gained and the plan of attainment, and through its activities among the people gave enormous impetus to a reduction of mortality that had been plodding along for twenty and more years before.

Almost seven hundred institutions for the treatment of tuberculosis—sanatoria—dot the country where scarce a score raised their roofs in the year of the National Association's birth. These institutions are the least disputed of organized effort's achievements. Except for insistent agitation—call it propaganda—originating from the *National* they had never seen the light. So much will be admitted. And what is their influence on tuberculosis?

Their "turn-over" approaches 150,000 patients a year. They therefore take from active life what must be at any time a large proportion of all people who put out tubercle bacilli, and keep these removed from infectious contact for periods of months to years. According to their annual reports, they close up many fountains of infection, as they free their patients' sputum from tubercle bacilli; while sanatoria for advanced patients, who die in institutions, quite often are breaking, for good, circles of contact that are among our most prolific and dangerous breeders of disease. Their more intelligent and responsible patients,— and these are the greater number,—they train in the ways of proper personal care and disposal of their sputum.

If there is a science of tuberculosis, it presents no fact more certain than that a most prolific cause of active tuberculosis is "massive" and frequently repeated, almost continuous, infection. To prevent this today there exists no instrument that may be compared with the sanatorium, residence in which brings about segregation, the healing of foci, and the education of the sick.

It is the writer's opinion that a fair case can be made out for the presumption that the very great reduction of mortality of the last few years—from figures already unbelievably low—is largely due to this unlooked-for prophylactic influence of sanatoria. These institutions

were created primarily to cure, not to prevent;—though more or less compulsory committal to sanatoria had long been advocated by way of prevention. While their number was small, and relatively inadequate, tuberculosis mortality jogged along about as usual, that is, declining leisurely and at times haltingly.[2] But as facilities began to approach requirements, as, in other words, a growing proportion of those ill with tuberculosis had their institutional needs supplied, the situation began to appear in an accelerated decline of mortality, which, after all, merely reflects a condition of fewer cases of severe tuberculosis, which is, in turn, a resultant of heavier infection. While the ideal, a bed for a death, is yet far from attainment[3] throughout the country, the tide of sanatoria is creeping up toward the line marked out by mortality, and it has now, for some time, reached a level of appreciable influence, which has become manifest in mortality returns.

To repeat:—There is so much less mortality because there is so much less severe disease, and so much less severe disease largely because there has been a diminution of infection, and not a small part of this diminution is being brought about by the withdrawal of an effective (as causing infection) proportion of the sick into institutions.

To repeat further:—The sanatorium mitigates and heals; the sanatorium prevents; and the sanatorium educates. It can be demonstrated mathematically that if every patient were put in a sanatorium at or before the first "show" of bacilli, and kept there as long as he expectorated bacilli, tuberculosis of the lungs would disappear upon the death of the last present host of bacilli—and, no doubt, long before that. Of course, such a "root and branch" weeding out of the tuberculous will never come near of accomplishment; but it is conceivable that, without being aware of it, we have caught up with and passed that stage in a delicately adjusted mechanism that marks equilibrium; that is to say, that so many tubercle bacilli are now behind institutional walls that not enough remain at large to infect mankind sufficient to maintain a given level of mortality,—*with, of course, environmental factors what they are.*

[2] Between 1902 and 1904 the death-rate rose from 185 to 201; in 1906 it was 180 and in 1907 only one point lower, at 179; from 1909 to 1911 it was almost stationary,—161 in 1909, 160 in 1910, and 159 in 1911,—as again from 1913 to 1915 (148 for 1913, 147 for 1914 and 146 for 1915); between 1916 and 1918 it rose from 142 to 150. Indeed, for the seven years, 1912 to 1918, the rate was practically stationary. The decrease between 1910 and 1918 was only 6 per cent.

[3] Exclusive of "health-resort states," six states in 1925 had sanatorium capacity of a bed per annual death. They were Massachusetts, Rhode Island, Connecticut, New York, Wisconsin and Minnesota.

In addition to the direct influence on the patient and on opportunities for mass infection, our sanatoria must contribute to the control of tuberculosis in another most effective way. There must be at least a half-million close associates of patients who every year, for the first time and as nontuberculous people, are learning something authoritative, be it ever so little individually, about the care and prevention of the disease. At the very least, the interest of a host of citizens is bound to be awakened and made more alive. Always new tens of thousands, with tuberculosis struck home, are brought to a pause,—and to ask, "What shall we do to avoid tuberculosis?"

It has been ever thus. But, whereas, not any too long ago these interested people were advised to look to their family trees, they may now receive sound instruction from both patients and the institutions themselves. And it is unbelievable that such collateral education on the part of sanatoria has not prevented or aborted clinical tuberculosis in thousands.

Numerous facts and signs support the assumption that the sum total of tuberculous infection is being markedly reduced. Limited space forbids their being set down here.[4] If the assumption is correct and if, as seems beyond doubt, the sanatoria constitute a major factor in cutting down infection, one direction of our future efforts against tuberculosis is plain.

Just as *now*, of all times, calls for the intensification and elaboration of the antituberculosis movement, so does it require the continued and further establishment of sanatoria. The more promptly we expand treatment for tuberculosis patients the nearer do we bring that day when it will be no longer required. Faster and faster will the deaths decline to meet the increasing number of beds. And we may be certain that if a situation of "a bed for every death" in tuberculosis ever does obtain throughout the United States, equilibrium will be almost immediately upset by a condition of more beds than deaths.

Such beds, vacant because of paucity of tuberculosis patients, will not—or need not—stand idle long. In preserving men from tuberculosis, or from typhoid fever, or from any and all infections, we save them

[4] Between 1898 and 1923 the tuberculosis death-rate for infants under two in New York City was cut in six and that for children under fifteen in four. This is surely first-class evidence of lessening opportunities for infection. For children the decline was 36 per cent for the period 1898–1910, and 62 per cent for 1910–1923. See Drolet, G. J., Tuberculosis in children. A review of the mortality during the past quarter-century in New York City, AMERICAN REVIEW OF TUBERCULOSIS, 1925, xi, 292.

to die from other diseases. So much is certain. And it is also indubitable that, as we escape death from the infections, we hold our lives in pawn for the day of dissolution—it is inevitable—due to the degenerative diseases. When tuberculosis and the other major infections are no longer of the earth, to take us from it, then most of us will yield to atheroma, to cardiac and to renal insufficiency, or perhaps to cancer. Whether we lengthen our span to one hundred or a thousand years, 'tis all the same—there is no other escape. Of man's six major maladies to-day only two, pneumonia and tuberculosis, are infections. Cancer and cardio-renal-vascular insufficiency make up the other four. And, as tuberculosis goes, and perhaps pneumonia and, conceivably, cancer, we must die of heart-kidney-arterial disease.

Therefore, a life snatched from tuberculosis is, according to the greater chances, a life delivered over to heart disease or one of its common congeners. Even though our work in tuberculosis is, as yet, not half-accomplished the other horn of the dilemma has already begun to sprout, as heart disease leaps ahead. But, at bottom, standard treatment for pulmonary tuberculosis is as good treatment as we can advise for chronic heart disease— or kidney or arterial disease. And, except for the element of infectiousness, the social problems of heart disease—the adaptation of patient to life's demands—are not far removed from those of phthisis, different though the average age and economic condition of patients may be. There is an "after-care," as well as an occupational therapy and vocational training, for "broken" cardiac patients just as there is for consumptives. And it is a reasonable forecast that the effective treatment of heart disease will in time be along lines familiar now to us in tuberculosis.

As for the prevention of heart disease, except as it arises out of syphilis or other infections, practically every precept laid down to keep tuberculous infection quiescent holds good for heart disease. Indeed, it has not been emphasized enough that the antituberculosis campaign must be preventing or delaying the appearance of much heart disease in thousands who have taken it to heart and guided their lives accordingly.

Sanatoria for tuberculosis, when available, can therefore always be turned over to the concomitant of the conquest of tuberculosis; as in time our communities will learn that they have exchanged one problem of preventive medicine for another, and will undertake the rehabilitation of the useful cardiac patient and the amelioration and prolongation of life of the doomed. Nor should these communities be depressed by

what might appear as a gloomy outlook. They may be obliged to exchange one problem for another,—there would seem to be no escape,—but they will have added to their civilization at least two decades of the productiveness of thousands.

According to current classification, there are at present only six really major diseases of adult life. We have seen that in 1924 *Diseases of the Heart* was first and *Nephritis* sixth, and that only 851 deaths, or a rate of less than one per cent, separated tuberculosis from the latter. Since we know that tuberculosis declined further in 1925 (*estimated* rate about 88), it is likely that it is now in sixth place.

After *Nephritis* in 1924 came *Congenital Malformations and Diseases of Early Infancy*, with 77,653 deaths, for a rate of 78.4; next, *Accidental Causes*, with 75,745 deaths (rate 76.5); and then *Diarrhoea and Enteritis*, with 34,482 deaths, of which 27,566 occurred in patients under two years of age. In a consideration of *diseases* of adult life, all these may be left out of account.

Tuberculosis therefore stands to-day at the foot of the list of major diseases. Next to it, as a fatal malady of adults, is the category *Diseases of the Arteries, Atheroma, Aneurysm, etc.*, to which *Cerebral Hemorrhage* (near the head of the list) is allied, and which was responsible for 23,278 deaths. To those who, but little more than a decade ago, cited 150,000 deaths as the annual toll of tuberculosis, it seems impossible that the disease can make the long descent from its present position to, say, 25,000 deaths a year. Yet, to traverse this gap means a reduction of about as many deaths from the figures of 1924 as these latter were below those of 1910. And if a comprehensive view of the forces at work means anything it should at least suggest that what happened between 1910 and 1925 can, with more reasonable hope of repetition, again take place between 1925 and 1940. If in the last-named year tuberculosis should cause only 25,000 deaths in the United States, its rate will be about 20 per 100,000, or no doubt on a par with deaths caused by automobiles, and the conquest of consumption will really be at hand. Surely, this is a goal to stir to action the laggard, or, above all, the volatile, who believe the job already done. To attain it will mean vastly harder work than that of the last decade.

A. K. K.

THE MODERN SANATORIUM TREATMENT OF TUBERCULOSIS AS APPLIED IN A LARGE GOVERNMENT INSTITUTION[1]

EARL H. BRUNS[2, 3]

The United States Government is now treating thousands of cases of tuberculosis from among the World War veterans. These patients are being cared for in large institutions scattered throughout the country, and an effort is being made to give them the best of treatment known to modern medicine. The results have probably not been as good as we had hoped for, because, in spite of our progress in the management and care of this disease, the outcome still depends largely on the character and coöperation of the patient, and ideal patients are, as always, few and far between. Following the discovery of the tubercle bacillus in 1882, by Robert Koch, a cure for tuberculosis has been sought for in the research laboratories of many countries. Although many specifics have been advocated, and hardly a year has passed when cures for this disease have not been broadcasted in the headlines of newspapers, we are still depending on the conservative methods of sanatorium treatment.

It is unquestionably true that tuberculosis frequently heals spontaneously, that is, without being discovered and without any treatment being instituted, but this does not mean that our present-day therapeutic efforts are superfluous and unnecessary. Even without being aware that active tuberculosis is present, we very often improve our mode of living or change our habits in a way which causes the disease to subside and become latent again. As, for instance, an individual is overworking, and, in consequence of which, a quiescent infection is lighted up, manifest by such slight constitutional symptoms as loss of weight, fatigue, weakness, nervousness, etc. Guided by his feelings, for the time being he gives up his customary diversions outside of working hours, goes to bed earlier or takes a vacation, and soon recovers a

[1] Published by permission of the Surgeon-General, U. S. Army.

[2] Lieutenant-Colonel, Medical Corps, U. S. A.

[3] Fitzsimons General Hospital, Denver, Colorado.

normal degree of health. The failures of spontaneous healing finally become manifest by more definite symptoms, and then the treatment generally accepted to-day as our only and best means of curing tuberculosis must not be delayed.

When an animal which has never come in contact with tuberculosis is inoculated with a sufficient dose of tubercle bacilli virulent for its species, it dies invariably of generalized tuberculosis. If, however, a minute initial inoculation is made, and after sufficient time has elapsed a second and much larger dose is given, aside from an early, acute and transient local reaction, the effect is very slight and the animal continues to live. It may eventually die of tuberculosis, but, if it is well fed and kept in its favorite environment, the disease may remain latent and finally heal. Compare the first animal with the young child or the uncivilized adult receiving for the first time a large infection, or, what amounts to the same thing, frequently repeated small infections; the result is similar. They die of acute or generalized tuberculosis, against which all treatment is of no avail. Tuberculosis, as it usually presents itself in civilized individuals, occurs in those who have survived the initial small infections, and have developed an immunity as the result of these infections. This immunity, while unable to eradicate latent tuberculous foci, is capable of keeping such foci latent and preventing the activities and spread of the tubercle bacillus. To do this, immunity must be kept at a high degree. If even a slight lapse occurs, tuberculous infections or latent foci may assume the proportions of disease. The whole problem of tuberculosis is one of immunity. We can only treat the disease through means which restore this immunity, or by assisting in every way the protective forces of the body itself against the tubercle bacillus. An improvement in the hygiene of living, increased rest, more fresh air and better food, all incorporated in the routine of sanatorium life, are the principles of treatment. While they may be inadequate in many cases, they are frequently sufficient to restore slight failures of immunity and turn the tide toward recovery.

The treatment of tuberculosis can best be carried out in a sanatorium. It can also be applied at home. While there are advantages in both methods, this much can be truthfully said about the sanatorium: It is the school of the tuberculosis patient and treatment should therefore be started this way. Following a certain period in a sanatorium, home treatment may be indicated, but if the patient is initiated into his treatment at home, it will require a greater effort on the part of both the

patient and physician to carry it through to success. The treatment of tuberculosis is an expensive proposition; it requires most careful nursing, attention to details, and often a change of climate. To copy with any degree of success sanatorium treatment at home, a private nurse and frequent visits of the doctor are required. In a sanatorium, one nurse can take care of a number of patients partly because every one is going through the same routine. At home the well members of the family lead a different life from that of the patient; family cares, worries, and annoyances are close at hand; it is more difficult to "chase the cure;" careful medical supervision is not to be had and details of treatment, each one perhaps trivial in itself, but important when taken as a whole, cannot always be carried out. The modern method of treating tuberculosis, which will be described later, and which demands repeated X-ray examinations, laboratory tests, more or less complicated therapeutic measures, and not infrequently surgical procedures, can best be employed in a hospital. It must not be ignored that for certain patients sanatorium life is depressing. The atmosphere of sickness, especially after a time, causes them to grow despondent and dissatisfied. They yearn for the home environment and crave home cooking. They have had their sanatorium training, and their disease has become quiescent. It will be more agreeable and easier for them to take their treatment at home. If home conditions are satisfactory and they have demonstrated ability and willingness to follow treatment, there comes a time when, even though they are not entirely well, it may be the best policy to have them complete their cure at home.

If asked to explain the modern method of treating tuberculosis, we would describe it best as it is carried out in a sanatorium. This paper will discuss the treatment as applied in the Fitzsimons General Hospital, which is one of the largest government tuberculosis institutions in existence to-day. It receives patients from the Army, Navy, Veterans Bureau, and United States Soldiers' Home. Built during the war, it is of the war-time semipermanent and pavilion construction, consisting of a central group of two-story buildings, all facing south, one long building containing the surgical wards, receiving wards, and wards for terminal cases, connected by closed corridors to the surgical pavilion, physiotherapy department, clinical laboratory, X-ray and dental clinics, patients' general mess-hall, and the various offices of the registrar, chiefs of services, chaplains, and representatives of the Navy and Veterans Bureau. Arranged to the east and west of this group there are twenty-

four wards facing south, widely separated, and staggered, so that each ward has a good outlook. In addition, there are many other separate

General Aeroplane View, Fitzsimons General Hospital

Heliotherapy Ward, Fitzsimons General Hospital

buildings, such as the administration building, post-exchange, officers' quarters, nurses' quarters, barracks for soldiers, and civilian employees,

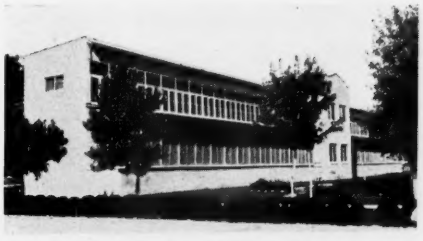
quartermaster buildings, supply buildings, utility buildings, storage buildings, Red Cross building, educational buildings, officers' club, etc. The hospital has a bed capacity of 1,857 patients.

Several years ago the writer had the opportunity of visiting a number

INFIRMARY WARD

OPEN-AIR PORCH, AMBULATORY WARD

INTERIOR OF GOOD-BEHAVIOR WARD



EXTERIOR OF GOOD-BEHAVIOR WARD

TUBERCULOSIS UNIT, FITZSIMONS GENERAL HOSPITAL

of tuberculosis hospitals in Germany and Switzerland, and certain questions concerning the treatment of tuberculosis were always asked the doctors in charge. On one point they all seemed to agree, namely, that the bed capacity of any one sanatorium should not exceed one hundred,

or at most two hundred beds, so that each patient could receive individual attention. In large institutions there would naturally be a tendency to treat patients in groups, and the personal contact between the patient and the doctor would to a certain extent be sacrificed to facilitate administration. Moreover, it would necessitate many different doctors handling and advising the same patient, thereby interfering with the continuity of treatment. While the method of treating tuberculosis in the smaller hospitals varies somewhat, the results obtained are about the same as individual, and continuity of treatment is employed equally well in all and can logically account for a large degree of success.

A tuberculosis institution with the capacity of the Fitzsimons General Hospital would therefore be a mistake if steps were not taken to correct this disadvantage of size. The hospital is conceived and administered not as one institution, but as a sanatorium centre made up of a number of units, each one more or less complete in itself. Each unit can be looked upon as a separate sanatorium in a centre or group of sanatoria under one administrative head, but exercising enough independence for all practical purposes and consistent with the best results in treatment. Thus, the disadvantages of a large institution are to a certain extent eliminated, at the same time meeting the necessities and retaining the benefits of a large institution. Among the advantages of the large tuberculosis hospital might be mentioned the services of trained specialists and consultants and the more highly developed and better equipped special diagnostic and therapeutic departments, hardly to be expected in the small sanatoria. Such a sanatorium centre contains a surgical unit, a neuropsychiatric unit, a general medical unit, an eye, ear, nose and throat unit, a central clinical laboratory, an X-ray department, physiotherapy department, dental clinic, educational and recreational department, and various administrative departments. Each tuberculosis unit consists of three wards, an infirmary ward, capacity one hundred beds, ambulatory ward, capacity eighty beds, and a good-behavior ward, capacity twenty-eight beds. The infirmary ward is very complete, contains a large diet kitchen, dining room, examining and treatment rooms, fluoroscopic closet, doctors' and nurses' offices, solarium, quiet room, heliotherapy platform, etc. In such wards provisions are made for heliotherapy, artificial-light therapy, pneumothorax treatments, eye, ear, nose and throat treatments, intravenous therapy, etc. In the diet kitchen, food is transferred from food-carts to steam-tables and a certain amount of cooking is done, such as the preparation of eggs, toast, and other simple

articles of diet. The ambulatory ward is almost as complete as the infirmary ward, except that no diet kitchen is provided, the patients eating at the general mess. The good-behavior ward is used for ambulatory patients who are very coöperative in their treatment and wish to be quiet. In a way, it is a reward for good behavior. In this ward each patient has a separate compartment containing a bed, chair, table and chiffonier.

Carrying out the same idea of continuity and individualization in treatment, an effort is made to keep patients under the same doctor during their entire stay in the hospital. The advantage of this can readily be seen. The doctor comes to know his patients intimately. He is not only fully acquainted with their physical condition, but learns their mental makeup, their peculiarities, their problems in life, and as a result is better able to treat and influence them. A closer and more personal interest thereby enters into the case. Furthermore, the work of the clinical director and the ward surgeon is simplified. It means that each patient receives continuous treatment under the best trained and qualified medical officers instead of passing under the care of a number, some efficient and others of only average ability.

As an illustration we will trace a patient during his period of treatment in the hospital. He is admitted through the receiving ward, where he remains approximately ten days, during which time all examinations are completed and his case is diagnosed. If manifestly tuberculous, he is then transferred to an infirmary ward, and when his condition permits is again transferred to an ambulatory ward belonging to the same group. During this time an operation may be required for which he goes to the surgical unit, returning to the same tuberculosis unit after recovery. If he leaves the hospital on furlough or is discharged as cured and later suffers a relapse, he goes to his former unit when he returns. One doctor with one or more assistants is in charge of each unit and follows the patients throughout their entire treatment. The treatment of tuberculosis is explained to each patient. They are told what they must do to get well, and the whys and wherefores. Certain checks are made to assure that they are taking their treatment properly.

If it is found that a certain individual is not coöperating, and, after the ward surgeon has failed by reasoning and persuasion to influence him, he is transferred to the enforced-treatment ward, which is constructed as the other infirmary wards, except that it is heavily screened and kept locked. When such a transfer is decided upon, the patient

must go, or leave the hospital. It is carefully explained to him that the transfer is not in the nature of punishment, but to enable closer supervision and to compel him to carry out the essentials of treatment. Here his clothing is taken away, but he receives the identical care and enjoys the same privileges as in the other infirmary wards. In course of time, he returns to his own ward again. By such a procedure disciplinary action is often avoided, and it is a policy more in keeping with the personal interest which the doctor must have in the recovery of his patient.

Tuberculosis cannot be treated by rule of thumb. To attain any degree of success, each case must be handled separately. The patient is not to be considered merely as a case. He is a human being, and must be dealt with from the psychical as well as the physical point of view. The successful tuberculosis specialist is one who is strict, but at the same time enthusiastic, conscientious and sympathetic. Such a physician inspires faith and willingness to coöperate on the part of the patient. In large government institutions there is a conglomeration of patients of different races, different nationalities, of various degrees of intelligence, and from diverse walks of life. Consider what difficulties this presents in the treatment of a disease like tuberculosis, which cannot be cured by the administration of medicine, but must be arrested by an improvement in the individual's habits, hygiene, nutrition, physical rest, and mental contentment. It can readily be seen how futile it would be to apply such methods to patients in groups and disregard entirely the personal side. In a great tuberculosis resort like Davos, Switzerland, there are many sanatoria, each catering to patients of different nationalities and diversified circumstances. For example, it has been observed there that Spanish patients do better if treated in a separate institution, because they are of a different temperament, desire a certain type of cooking, and cannot endure intense cold. The same is true of other nationalities. Before entering into the discussion of the treatment of tuberculosis, it is therefore fitting that the necessity of individualization be stressed, and the organization and policy of conducting the tuberculosis service at the Fitzsimons General Hospital are endeavors to correct this defect in handling large numbers.

Rest, both local to promote fibrosis, and general to lower demands upon the reparative powers of the body and permit recuperation; an abundance of pure air with improved oxygenation; and sufficient good food well assimilated, are what constitute the tripod of treatment in tuberculosis. This is what cures our patients in the majority of in-

stances. We depend upon it for results, and, whatever else we do, this basic treatment must not be neglected. The difficulty lies in obtaining patients with sufficient character or in being able to influence them so that the three essentials of treatment will be carried out properly and continued long enough for permanent results. Rest can only be relative, but the more absolute it can be made, the better. Patients must not only be taught to stay in bed but how to relax properly. Rest in bed should be continued until constitutional symptoms have disappeared and until the X-rays show a clearing up of inflammatory reactions. Fresh air can best be secured on open porches. In large government institutions, it is very difficult to keep the ward windows open during cold weather. They are being continually closed by some patient, and it requires the closest supervision over ventilation on the part of the hospital personnel to carry out this phase of the treatment. If patients can be induced to "chase the cure" on the open porches, this difficulty does not arise. The necessity for fresh air must be constantly stressed by the doctors and rules regarding it never relaxed. Occasionally there are patients who cannot endure cold for various reasons. During the winter they should be treated in private rooms. Cold must not interfere with rest and relaxation, but with proper clothing, bedding and hot-water bottles, this need not occur. Patients soon become accustomed to pure air and cannot endure close rooms. Nutrition also presents its problems. There is a tendency on the part of many patients to overeat. They live to eat, and if they gain weight it is considered an infallible sign of improvement. Milk drinking and nourishment in between meals are not good practice, except when specially indicated. Stuffing should be discouraged at all times. Undernourished patients with anorexia have to be urged to eat and an effort made to induce them to take a certain number of calories by frequent feedings, highly nutritious food such as eggs and milk, and dishes prepared to their liking. After their normal weight is reached, moderation in eating should then be advised.

When the tuberculous lesions have become quiescent and constitutional symptoms have disappeared, graduated exercise becomes a necessary part of the treatment. The reason for this can readily be explained. The tubercles have now acquired a fibrous investment. The spread of tubercle bacilli which one phase of treatment, especially rest, has endeavored to prevent, is no longer a menace. We can gradually apply the beneficial effects of exercise without any of its dangers. The body has now recuperated in strength and can respond to exercise without

fatigue. Metabolism is improved. In addition, exercise, if properly applied, causes slight focal reactions around the tubercles, which now, instead of being harmful, spreading tubercle bacilli and their products and leading to caseation and necrosis, are beneficial, causing increased fibrosis and repair. At this stage of the treatment, however, patients often become careless and difficult to restrain. They are too prone to abuse any liberties and soon overstep their limits. Feeling well, the need of caution is not so apparent to them. Unfortunately, the doctor also fails to do his part and may relax his supervision. Ambulatory patients feel that they are entitled to passes. If passes are granted, graduated exercise ceases to be graduated, because, while on pass, the patient will not make his exertions conform to the stage of his graduated exercise. Exercise is first prescribed in the form of sitting up in bed or in a reclining chair, toilet privileges, then graduated walks and later manual labor of some variety, such as cleaning work in the wards or about the hospital grounds, or training in the curative work shops. In prescribing exercise in tuberculosis, the doctor must be familiar with the patient's physical condition and the character of his lesions. Cases of nodose or fibrous tuberculosis, with little or no perifocal reaction, do better on graduated exercise. It is a mistake to keep such patients continually at rest. The effect of exercise must be carefully watched. Symptoms must be checked and serial X-ray plates studied. As a rule, reactions and healing will be manifested by changes in symptoms and X-ray shadows, long before detected by physical signs. The modern treatment of tuberculosis demands as a guide the study of well-made serial X-ray plates.

The rôle of climate in combating tuberculosis is a much-debated subject. Everything else being equal, it cannot be denied that a dry climate with seasonal changes and a maximum amount of sunshine, like that of Colorado, is favorable for the cure. While the Army recognizes the importance of climatology and has always located its tuberculosis hospitals with this in view, the part which climate plays in recovery from tuberculosis has never been exaggerated or emphasized too strongly, as it misleads patients and causes them to neglect the basic principles of treatment.

The care of tuberculosis in the adult often has the great disadvantage in that while patching up his lesions, we make a neurasthenic of him. A child can be treated without this drawback. Sanatorium management has come to recognize more and more the mental side of the tuber-

culous patient. Useful bedside occupational work, certain forms of harmless diversions and light amusements, such as moving pictures, radio, musicales, and games of various sorts are permitted as the disease becomes arrested. At the Fitzsimons General Hospital the moderate use of tobacco is not discouraged. Gambling and all forms of dissipation are forbidden and offenders subjected to disciplinary action. Educational work is carried out, both at the bedside and in the class-rooms of the educational department. As long as it is compatible with treatment, everything must be done to keep patients satisfied and contented, and their time fairly well occupied. As the disease becomes quiescent and the patient is allowed to get up and around, the problem of his rehabilitation is to be considered. He must be made to feel that his days of usefulness are not over and that the plan of treatment is eventually to restore him to his former occupation or some new vocation more suitable to his physical condition. In handling government patients, we must strive to steer them away from the path of the chronic pensioner and pauperism. The pension and compensation systems and the prolonged treatment necessary in tuberculosis are largely to blame, and not so much the patients themselves. If they can be made to see from the very beginning that the whole plan is to ultimately restore them to health and full, or at least partial, working capacity, that in time they will probably have to make their own living, and that they must save and prepare for this eventuality, many will be rescued from lives of idleness. The time to bring such influence to bear is at the beginning of hospitalization.

Rather closely connected with the hygienic treatment of tuberculosis is heliotherapy. Radiation of the diseased part and the whole body with the sun's rays or artificial light from mercury vapor and carbon arc-lamps has recently come into fairly common use, especially in forms of extrapulmonary tuberculosis, such as tuberculosis of bones and joints, glandular tuberculosis, genitourinary tuberculosis, tuberculous peritonitis, skin tuberculosis, etc. Heliotherapy can also be employed with good results in pulmonary tuberculosis, provided cases are properly selected. Ambulatory patients with fibrous or nodose lesions, with little or no inflammatory reaction, should be chosen, and the treatment given very cautiously. Without going into details, the exposures must be graduated at first, increasing the time and the area exposed until the whole surface of the body, with the exception of the head, receives two to three hours of the sun's rays, or twenty to thirty minutes under the lamp each

day. The action of light treatment in pulmonary tuberculosis is largely due to the improved metabolism and the mild focal reactions produced. In taking sunbaths, patients are also receiving air baths, which are given much credit by some for the good results obtained. Sun and air baths naturally harden the patients and enable them to live more comfortably out-of-doors. While provision should be made for applying heliotherapy and light therapy on all wards, in a large institution a heliotherapy ward is advisable. At the Fitzsimons General Hospital each tuberculosis unit has a heliotherapy platform and a mercury-vapor lamp. In addition, a separate heliotherapy ward or unit is provided, with an orthopedic surgeon in charge. To this ward all cases of extrapulmonary tuberculosis are sent. Certain selected pulmonary cases are also treated on the heliotherapy ward, the plan being to make this unit the centre for all heliotherapy activities in the hospital, where the technique and results of this therapeutic procedure can be studied as an incentive and guide to its application in other parts of the institution.

In treating tuberculosis of bones and joints we depend much upon immobilization and local rest. Pulmonary tuberculosis heals with astonishing rapidity when the diseased lung is placed at rest. Of course, quiet and relaxation in bed afford much rest for the lungs, likewise our various means of controlling cough, but to secure rest, absolute or approaching the absolute, other methods have to be resorted to, such as postural—lying on the affected side, strapping the chest with adhesive plaster, Sewall's chest belt, and certain braces and appliances for immobilizing one side of the chest. These procedures are all helpful. Based on the rest principle, collapse therapy has been developed. Artificial pneumothorax is our simplest and best method of placing the lung at rest by means of collapse. This method of treatment has been gradually perfected. To-day, by resorting to low pressure and selective collapse, it can be used with less danger and fewer complications, and its employment in earlier cases and in bilateral involvements is more frequent. In selective collapse only the diseased lobe is completely compressed and fixed, while the remaining lobe or lobes are merely kept surrounded by a thin cushion of air and are permitted to function. Often to obtain a selective collapse, the whole lung may first have to be completely compressed in order to separate adhesions, and then the selective collapse be obtained by selective reëxpansion. A diseased lobe, providing it is freed from adhesions, being less elastic, can be compressed with less pressure. On this fact selective collapse is based. It is con-

ceivable, and in certain selected cases it is practicable, by the application of the principle of selective collapse, to employ pneumothorax therapy bilaterally. Pneumothorax treatment is not without its dangers, such as air embolism, spontaneous pneumothorax and pleural infections. It is not a therapeutic measure to be considered lightly, either by the patient or physician. At the beginning pneumothorax patients should be made to understand that "chasing the cure" will still be necessary.

More recently surgery, in the form of phrenicotomy and thoracoplasty, has come into use for collapse of the lung, usually in cases in which pneumothorax cannot be induced on account of pleural adhesions, or is contraindicated for some reason.

Phrenicotomy, exairesis of the phrenic nerve, or unilateral paralysis of the diaphragm by excising a section of the phrenic nerve, where it lies fairly accessible in the neck, is equivalent to injecting several hundred cubic centimetres of air into the lower pleural sac. The diaphragm becomes elevated and fixed on the operated side. This effect can best be determined by fluoroscopic examination, comparing the two sides when the patient takes a deep inspiration. It is very remarkable at times how rapidly lesions in the lower part of the lung clear and fibrose, following this simple operation. Basal cavities, 3 to 4 cm. in diameter, have been known to heal within a few months. Phrenicotomy is employed as a preliminary and adjunct to artificial-pneumothorax treatment and thoracoplasty, or alone for tuberculosis and cavitation of the lower lung.

Thoracoplasty or extrapleural paravertebral resection of the ribs immobilizes and compresses the lung. By removing portions (4 to 14 cm.) as near the spine as possible, the ribs are not only shortened but the anterior ends drop down and assume a more oblique position, in which position they become fixed by bony bridging, thereby narrowing and immobilizing the chest on the operated side. Thoracoplasty is indicated mostly in advanced lesions of the lung, when pneumothorax cannot be induced on account of pleural adhesions. Unfortunately, it is a last-resort major operation, and is usually performed on patients who are poor surgical risks. If we consider that it is almost always the only chance which stands between the patient and death, the high mortality does not condemn it entirely as a therapeutic aid in pulmonary tuberculosis. Statistics show the operative mortality to be from 20 to 25 per cent, but they also show that 50 per cent of patients are saved by it who would likely die within a short time. The mortality rate will prob-

ably be materially reduced by improved technique, by exercising more care and skill in selecting cases, and by employing it earlier in the disease.

It might be well to discuss briefly some of the graver complications and dangers of thoracoplasty. Shock due to the character and extent of the operation can often be avoided by resecting only as many ribs as the condition of the patient permits. Haemorrhage can be minimized by careful technique, especially placing the incision far enough away from the midline so as to avoid the thick paraspinal muscles. If shock due to loss of blood occurs, blood transfusion by the direct method or the administration of normal salt solution by hypodermoclysis or by vein is usually effective. The cause of most of our deaths has been a profound toxaemia, which usually develops within twenty-four hours and leads to a fatal termination during the first week following the operation. This condition is manifest by fever, prostration, cardiac depression, jaundice, cyanosis, and ileus. It has been attributed to various causes, such as auto-tuberculization, that is, a squeezing into the lymph channels of the toxic products of the tubercles; a locking up in the lung of the secretions of cavities and diseased bronchi; a toxic absorption from the wound due to coagulation necrosis or the gangrene of traumatized tissue. When toxic substances are forced into the lymph circulation, a lighting up of quiescent lesions in the opposite lung may take place, simulating a severe tuberculin reaction. The same effect may result from vascular congestion in the uncompressed lung, due to changes in circulation from sudden displacement of the thoracic viscera, chest flutter and pendulum breathing occurring during and after thoracoplasty. Patients are unable to raise sputum for several days following the operation because coughing is painful and the operated side is not under control. It is conceivable that this retention of sputum may give rise to toxaemia. In resecting the ribs, the muscles, especially the intercostal, and fasciae are torn and contused, and before sutures are applied we have a long and wide ragged wound, the like of which is seldom seen in any other surgical procedure. Probably of some significance in this connection is the fact that among our cases the type of sapraemia just mentioned has occurred more often in well-nourished and well-muscled patients. The thin, flat-chested consumptive, even though acutely ill, as a rule does better in this respect than the more robust individual. Thus we have a type of operation prone to be followed by considerable toxaemia, due to the absorption of the toxic products of the tubercle, pulmonary inflammatory secretions

and traumatized tissue. This occurs in an individual with degenerated and damaged viscera, whose powers of elimination and recuperation are weakened.

To avoid or minimize this toxaemia, a two or more stage operation should be the the rule, light postoperative strapping, more careful dissection and suturing should be performed, and a drainage tube properly inserted. Based on the theory of locked-up secretions and auto-tuberculization, the patient should be encouraged to drain his cavities and cough out all sputum just prior to operation, no morphine should be administered before or after the operation, and the position of the patient should be changed at regular intervals. Coughing and the raising of sputum should be resumed as soon as possible. A preliminary pneumothorax, when possible, even though only partial, or a phrenicotomy and a multiple-stage operation by gradually changing the intrathoracic circulation and visceral relationship, may aid in preventing or lessening this complication. For the same reason, strapping the chest should only be supportive at first and later gradually tightened. Aspiration tuberculous bronchopneumonia into the base of the opposite lung is not a common complication, but according to our experience in resecting ribs from above downward, it is very likely to occur into the base of the lung being collapsed. Consequently, we make it a rule to always stage the operations from below upward. The general impression that the operation is very mutilating is incorrect. When clothed, no deformity is apparent. The indications for thoracoplasty are about the same as those for pneumothorax. Lesions in the better lung are no contraindication, providing the lung has good function and the involvement is of the chronic type and not too extensive. We have repeatedly observed and have been impressed with the rapid healing of tubercles and the shrinkage of cavities in the good lung as a result of the operation.

Thoracoplasties may be complete or partial, upper or lower stage. Complete thoracoplasty is usually performed in two or more stages, resecting the ribs from below upward. The upper-stage thoracoplasty can be used to obliterate large cavities in the upper part of the lung, and lower-stage to splint active lesions and obliterate cavities in the lower part. Lower-stage thoracoplasties are very effective. The upper-stage usually require the posterior resection of the upper seven ribs supplemented with anterior resection to accomplish its purpose.

Thoracoplasty is also employed in tuberculous pyopneumothorax to reduce to a minimum the size of the affected pleural sac. In such cases the

rib resection must be wide and, if the pneumothorax is complete, it may have to be followed by continuous tube-drainage, thoracotomy, and eventually further plastic procedures to completely obliterate the pleural cavity. Thoracoplasty is valuable in advanced fibrous cases with large cavities. These cases no longer obtain much help from hygienic treatment. The maximum benefit has been attained through fibrosis. The cases are suffering no more from tuberculosis *per se*, but from the destructive and secondary effects of the disease. It is principally a question of obliterating large cavities, preventing haemoptysis, eliminating the toxaemia of cavities, and saving the patient from ultimate amyloidosis or other degenerative visceral changes. Pneumothorax cannot be employed on account of dense pleural adhesions, and thoracoplasty is our only hope.

Apical pneumolysis, with various methods for filling the extrapleural space, is associated with shock and postoperative pain, and has not proved very efficacious. This operation in itself is of quite limited usefulness. It can be employed for obliterating large upper-lobe cavities, but at the best it causes only a temporary obliteration, and in the end the cavity is merely slightly reduced in size. Likewise, the external drainage of cavities is restricted to rather rare conditions in which old cavities with mixed infections cannot be emptied readily by posture and coughing, and act very similar to a pulmonary abscess.

Tuberculin therapy plays a somewhat circumscribed part in the treatment of tuberculosis, especially in our government institutions. Its applicability is more to ambulatory cases just prior to their discharge from the hospital. Given by the "stealthy method," that is, beginning with very minute doses and gradually increasing until large doses are tolerated without reaction, it has a triple value of perhaps increasing fibrosis, maintaining the interest of the patient in his treatment with a closer relationship between the doctor and the patient, and as a test of the degree of fibrous investment around the tubercle. A tolerance to large doses can be interpreted in this light or as an index of high immunity (positive anergy of Hayek). There are many different tuberculins, but the form used is of minor importance. The question of dosage is of greater consequence. Tuberculin is no doubt very helpful in combating tuberculosis, but its use is rather impracticable and limited on account of the difficulty of regulating the dosage for any individual case.

Many specifics, chemical and serological cures for tuberculosis, have

been advocated from time to time. Looking back, we can recall how some of these have received serious consideration for a while, and then are heard of no more. After having observed repeatedly the fate of such remedies, it is wise to view all with suspicion, even though at first favorable reports are received. Physicians are frequently misled by the psychic effect of a well-advertised cure, and false hopes do our patients much harm. It is the best policy to cling to the established methods of treatment and never permit therapeutic fads to creep into our institutions.

The scope of this paper will not permit of a detailed discussion of the symptomatic treatment of pulmonary tuberculosis and the treatment of the various common complications. Briefly, we can state that the relief of cough, haemoptysis, pleurisy pains, night-sweats, anorexia, insomnia, digestive disturbances, etc., presents no innovations. Of the common complications, tuberculous laryngitis is best treated by absolute rest of voice, electrocautery, and heliotherapy in certain select cases. Local antiseptic and caustic applications are of no value. Tuberculous enteritis can be helped, at least symptomatically, by diet, heliotherapy and calcium chloride intravenously. Contrary to the general conception, tuberculous enteritis does heal, as proved by autopsy findings, and treatment is not always futile. Fistula-in-ano should be treated by heliotherapy, and, if the patient's condition permits, complete excision of tract and packing with iodoform gauze. In spontaneous pneumothorax, we may first have to combat shock, cyanosis and dyspnoea, and then turn our attention to the closure of the fistula and the prevention of empyema. The control of cough and quiet must be secured, and, as soon as the patient's condition will permit, a needle inserted and manometer readings obtained. By maintaining a positive pressure, if necessary, through the introduction of more air, the fistula may be kept closed. Valvular fistulae usually demand the insertion of a rubber tube into the pleura and connecting it with another tube leading into a bottle containing an antiseptic fluid. The advent of empyema brings into consideration the treatment of mixed-infection tuberculous pyopneumothorax by thoracoplasty, tube-drainage, and thoracotomy. All forms of extrapulmonary tuberculosis respond well to heliotherapy. In such cases the hygienic treatment is the same and as necessary as in pulmonary tuberculosis.

Compared with a decade or more ago, much is now being done for the chronic case of tuberculosis. Gradually our therapeutic methods have

been perfected and enhanced, some of the new developments in the treatment of this disease being mere ripples and others large waves on the tide of progress, until to-day the mortality rate, not only in the early, but also in the advanced cases, has been greatly reduced, and much more real hope added to the euphoria of the patients. The development of the X-ray and the employment of serial roentgenograms as a guide to treatment have been a wonderful help. It has permitted us to visualize pathological changes occurring in the lungs, which formerly we could only roughly infer through physical signs and symptoms. The necessity of large government institutions for the care of tuberculous ex-soldiers has threatened a handicap in obtaining the best results from treatment, but this has been largely overcome by certain policies of administration conducive to a closer personal touch between the doctor and the patient and facilitating continuity and individualization in treatment.

A CASE OF LIMITED MILIARY TUBERCULOSIS[1]

J. VINCENT FALISI[2,3]

The following report is submitted not because of any unusualness in symptomatology or outcome, but because of the insignificance of what is believed to have been the focus of a hematogenous dissemination, and because this dissemination was apparently limited to the choroid plexus and base of the brain.

Extract from Clinical Brief

No. 421: Patient is a white female, thirty years of age, a trained nurse with war service. Father died of nephritis.

Present illness: Patient very ill when admitted to this hospital and direct history not obtained. The following information was obtained from her brother: So far as is known, patient was well until three years ago, when during a physical examination tuberculosis was diagnosed and patient sent by Veterans Bureau to Saranac Lake, where she remained for about three years, being discharged as an arrested case. About three weeks previous to entering this hospital, patient complained of dizziness, headaches and cardiac discomfort, lost weight and had a poor appetite. At this time she had a physical examination, but nothing of importance was found. Shortly after this, while automobile riding, she noted a sense of oppression in her chest, which increased to a severe dyspnea. The following day she began to vomit, and from then on she could not retain food, and complained of headache. She was admitted to this hospital July 20, 1925. At this time she complained only of severe headache and constipation. She looked very ill and emaciated, tongue badly coated, slight sordes on teeth. Chest examination was negative except for a few râles elicited over right upper lobe. Patient soon became semicomatose and delirious. After two days she developed a muscular weakness of right extremities, some rigidity of neck, partial Kernig's sign in both legs, and exaggerated reflexes. She became progressively worse, and died July 29, 1925.

Résumé of Laboratory Findings

Urine: Catheterized specimen for culture, July 23, negative. Catheterized specimens, July 28 and 29, alkaline reaction, showed considerable albumen,

[1] Published by authority of the Surgeon-General, U. S. A.

[2] Major, Medical Corps, U. S. Army.

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negative for sugar, contained white blood cells and many red blood cells, and were very cloudy.

Sputum: Not obtained.

Blood counts: 10,350 white corpuscles, polymorphonuclears 85 per cent, on date of admission; July 21, 11,100 whites, with 80 per cent polymorphonuclears; July 25, 14,850 whites, with 90 per cent polymorphonuclears; July 26, 24,500 whites, with 89 per cent polymorphonuclears; July 27, 30,600 whites, with 91 per cent polymorphonuclears; July 28, 51,500 whites, with 99 per cent polymorphonuclears; July 29, 31,550 whites, with 95 per cent polymorphonuclears.

Blood cultures: Negative.

Spinal fluid: July 25, 1925, when removed, was under considerable pressure, and showed cell count 243, with 90 per cent small lymphocytes, and positive globulin, Noguchi and Nonne-Apelt. Colloidal gold test gave the following curve: 1123310000. Spinal fluid, July 27, still clear, but not under pressure; cell count 233; other findings similar except that sugar was negative.

X-ray report: July 21, 1925: *Chest:* Single bedside films. Bony thorax, diaphragm and mediastinal contents appear normal. Upper portion of right lung shows evidence of mottling and infiltration, and the same condition is apparently present in the left apex and possibly the first interspace. No evidence of acute pathological changes noted. Remainder of lung fields appear fairly clear. July 27, 1925: no appreciable change noted. (a) No chest complications present. (b) Oldest lesion is in right upper lobe. (c) No true clinical evidence of pulmonary activity can be stated. (d) No secondary lesions are present. (e) Heart is negative.

Résumé of abdominal findings: Upon admission the abdomen was scaphoid. This remained so until about July 27, when it became distended and quite tender. The outline of a distended bladder could be seen on lower abdominal wall. After catheterization distention continued in spite of enemata, and a complicating peritonitis as a cause of the marked leucocytosis cannot be ruled out. Relatively severe cystitis is present and might also account for leucocytosis.

Résumé of neurological findings and interpretation: Patient's illness began with vomiting, which persisted several days. Headache apparently occurred very early, but was not definitely localized as predominantly frontal or occipital. The last two days before death headache must have been relatively severe. There was stiffening of the neck, bilateral Kernig's sign, exaggerated superficial and deep reflexes, and some weakness of the muscles of the right extremities. Toward the last Babinski was positive, bilateral. With these symptoms, clear spinal fluid with count as high as 240, positive globulin, negative sugar, and history of pulmonary tuberculosis, it is believed almost

certain that tuberculous meningitis is present. The extreme leucocytosis may be accounted for by the severe cystitis or possibly peritonitis.

General clinical resumé: Final diagnosis: 1. Tuberculosis, pulmonary, chronic; activity undetermined, upper lobe right lung; lesions discrete and confluent, fibrous and caseofibrous above 2nd rib, right. 2. Meningitis, tuberculous. 3. Observation for peritonitis. 4. Cystitis, acute, cause undetermined.

Immediate cause of death was the complicating tuberculous meningitis.

Extract of Postmortem Findings

Heart and pericardium: Pericardium is smooth and glistening and contains about 30 cc. of straw-colored fluid. The heart, externally and on section, presents nothing pathological.

Aorta: Normal.

Lungs: Left: Pleural adhesions are found at the apex and laterally, but these are easily separated. The lung crepitates throughout, and no nodules or consolidations are felt. The apex is puckered by an old fibrous reaction. On section, small calcified lesions are seen at the apex; the rest of the lung is apparently free from lesions. *Right:* This is adherent at apex and posteriorly by easily separated adhesions. External examination of this organ is negative, except for a moderate puckering at the apex. On section, small old lesions are seen at the apex. One of these, about 1 cm. in diameter, shows a distinct perifocal reaction with some softening in the centre, and a surrounding dark red areola. There is no evidence of miliary tuberculosis, or other lesions in this organ.

Liver: Is small and soft. No pathological changes are observed on external surface. On section the markings are distinct. No tuberculous lesions are seen.

Spleen: This organ is very small, but is otherwise negative.

Adrenals: Negative.

Pancreas: Negative.

Kidneys: Capsule strips easily. On section the cortex is very pale, with dark red medulla. No tuberculous lesions are seen.

Bladder: This organ is distended and deeply congested and is very easily ruptured in its removal. It contains a very foul-smelling, purulent urine. The mucous membrane is eroded, and in areas the bladder-wall very closely approaches a gangrenous condition.

Uterus: Is small and contains a small clot.

Ovaries: Are normal in size and appearance, and the right shows a recent rupture of a follicle.

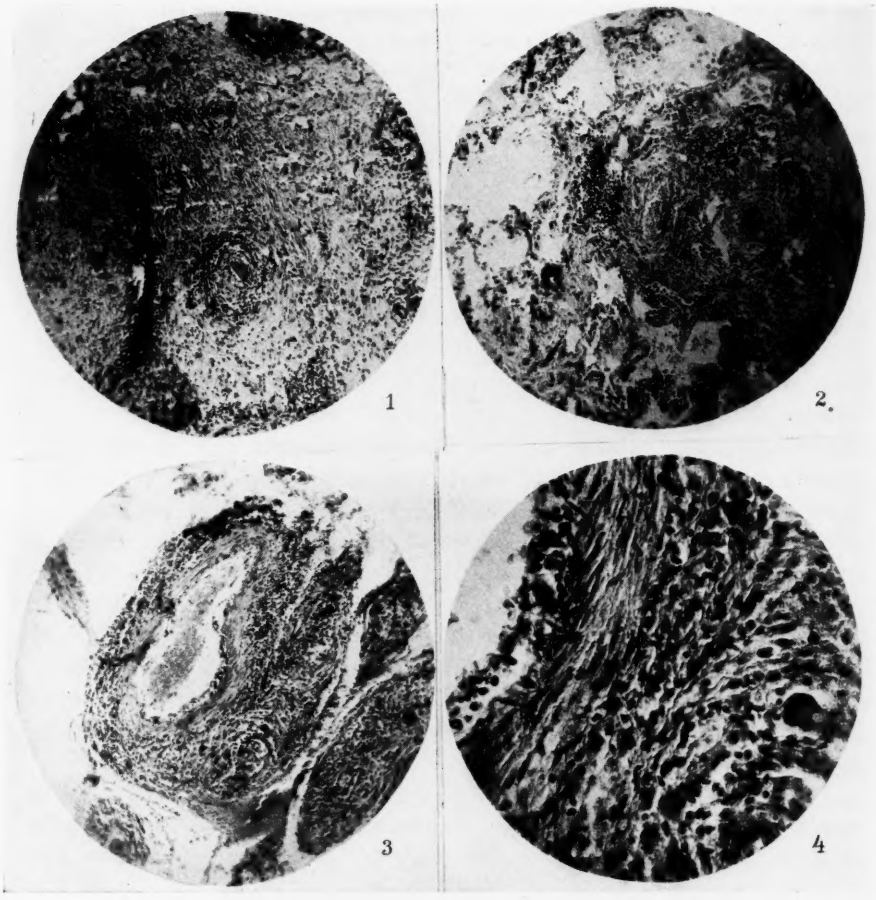

FIG. 1. FROM LUNG, SHOWING OLD LESION ON THE LEFT WITH DEFINITE LINE OF DEMARCATION, AND A YOUNG TUBERCLE IN THE SURROUNDING FIBROUS TISSUE

FIG. 2. SHOWING A BLOOD-VESSEL IN THE CENTRE SURROUNDED BY A TUBERCULOUS REACTION; AND A YOUNG TUBERCLE TO THE RIGHT; A REPRESENTATIVE ARRANGEMENT OF THE PROBABLE PORTAL OF AUTO-INOCULATION

FIG. 3. CHOROID PLEXUS, SHOWING BLOOD-VESSEL AND A YOUNG TUBERCLE—THE PORTAL OF EXIT

FIG. 4. AN ENLARGED VIEW OF FIGURE 3, SHOWING VESSEL TO THE LEFT AND A GIANT CELL TO THE RIGHT

Tubes: Negative.

Stomach: Is ruptured in manipulation and otherwise shows evidence of autolysis.

Intestines: Negative.

Brain: On removal of skull-cap the vessels are seen to be markedly congested.

Examination of superior surfaces is negative. The base of the brain shows a plastic exudate covering the cerebrum near the medulla, also the pons, medulla and cerebellum. Small tubercles are observed and a smear reveals tubercle bacilli. Section of brain shows nothing of interest other than a moderate dilatation of the lateral ventricles.

Microscopic Study

Pancreas: Negative.

Liver: Moderate congestion.

Kidneys: Cloudy swelling; postmortem changes.

Adrenals: Negative.

Lungs: Left: Acute inflammatory reaction. *Right:* Numerous small tubercles in an area of old fibrosis. This section was taken from lesion showing a definite perifocal reaction (see figs. 1 and 2).

Spleen: Capsule and trabeculae thickened.

Choroid plexus: Many small tubercles. Acute reaction surrounding blood vessels (see figs. 3 and 4).

Brain: (Base) Pia shows an acute, inflammatory reaction, but no tubercles are seen. However, tubercle bacilli were demonstrated in a smear from the exudate.

RÉSUMÉ

It is rather unusual to find a miliary tuberculosis so limited, no organ other than the brain being involved. It is believed that the original focus is the small, apparently active lesion in the right lung, described above.

Since no lesion, pyogenic in character, is observed other than the marked cystitis, this is believed to have been the cause of the marked polyleucocytosis.

RECOVERY FROM TUBERCULOUS MENINGITIS

Report of a Case

B. T. McMAHON[1]

Recovery from tuberculous meningitis is sufficiently rare to warrant recording its occurrence. Cramer and Bickel (1), in reporting a case, give an exhaustive survey of the literature and a critical review of the 46 authentic cases which had a favorable issue.

A woman, single, white, stenographer, age 28, was admitted to Loomis Sanatorium, August 5, 1923. She had had "pneumonia" twice in childhood and an appendicectomy in 1922. There was nothing else of importance in the past history.

She had an attack of influenza during the epidemic of 1918. Since then she had not felt quite up to her usual health, although she continued to work. Following the appendicectomy in June, 1922, she noticed she was losing weight and had little appetite. The work which formerly was easy now left her tired and weary in the evening. A slight hacking cough developed, with occasional scant sputum. This was examined for tubercle bacilli in November, 1922, but was found to be negative. As these symptoms persisted, gradually getting worse, and as there was slight evening elevation of temperature, she discontinued her work and entered Ridgewood Tuberculosis Camp, South Carolina, March 15, 1923. Shortly after admission she had blood-streaked sputum for a few days, apparently not attended by febrile reaction. She was discharged July 28, 1923. On admission to Loomis Sanatorium she appeared alert and intelligent, under medium height and considerably overweight. Granular breathing, with slightly prolonged expiration at the right apex, the only adventitious signs, was not sufficient to warrant a diagnosis of clinical pulmonary tuberculosis. The physical examination was otherwise negative, except for slight tenderness and the impression of a palpable mass in the right iliac fossa. Tubercle bacilli were not demonstrable in the sputum. Wassermann and tuberculosis complement-fixation tests were negative. The blood-lipase index was 0.86 (normal 1.00). Stereo-roentgenograms showed very slight infiltration of the upper part of the right upper lobe with a few peritruncal deposits scattered below this. The appearance was suggestive of tuberculo-

[1] Loomis Sanatorium, Loomis, New York.

sis. The urine was normal. At the monthly sputum examination tubercle bacilli were demonstrated on two occasions, September 12 and October 10, 1923.

The patient was allowed to work three hours a day as a stenographer, devoting the remainder of her time to rest on her porch. On November 10, 1923, without seeking medical advice, she married another tuberculous patient in the Sanatorium. On November 25, 1923, she complained of headache and malaise, and had a temperature of 100.4°F. (rectal). In the next few days the headache became worse and the patient became irritable, somnolent and drowsy. Nausea and vomiting were troublesome and increasing in frequency. The vomiting was of the projectile type. The pupils were equal, reacted to light and accommodated. Superficial and deep reflexes were exaggerated. Kernig's sign was positive and Babinski's sign negative. There were no sensory disturbances or cranial nerve paralyses. The retinal vessels were engorged, and there was central elevation of both optic discs. The head was slightly retracted and painful on flexing the neck. On December 3, 1923, the temperature rose to 103°F. (rectal). For the following fifteen days the maximum recorded daily temperature varied between 103° and 105°F., and then declined by lysis. The pulse rate was increased, but was slow in proportion to the fever. From this time until October 25, 1924, there were five acute exacerbations of fever and other symptoms, separated by afebrile periods. The dates of these were: January 10 to February 6, March 5 to March 16, April 4 to April 20, May 2 to May 25, and October 22 to October 25. Shortly after the onset of these symptoms a lumbar puncture was done, and this procedure was repeated from time to time. With one exception (see laboratory tests) only small amounts of fluid were obtained, apparently a condition of hypotension existing. After an afebrile period of seventeen days, a lumbar puncture was made on April 4, 1924. This was promptly succeeded by a sudden rise in temperature which persisted for three weeks, the daily fastigium varying between 103° and 105.4°F. (rectal). Lumbar punctures were then discontinued. Each time for the duration of the fever the patient gradually lapsed into coma, from which she could with difficulty be aroused. There was no loss of sphincter control. During the intermissions she regained consciousness, but the pain and retraction of the head, photophobia, nausea and occasional vomiting persisted. A transient right-sided partial hemiplegia, involving the arm, leg and trunk, developed, following the febrile period of January 10 to February 6, 1924. From October the temperature was normal except for a few intermittent daily elevations of one or two degrees. Symptoms gradually cleared up, and on discharge from the Sanatorium on December 15, 1924, symptomatic cure was evidently complete. Further encapsulation of the pulmonary lesion had occurred. At the present time, September, 1925, the patient reports that she is well, living with her husband and doing her own housework.

Laboratory tests: The first lumbar puncture was done on December 9, 1923. A small amount (2.5 cc.) of a highly bloody fluid was obtained. This fluid was inoculated into guinea pig 173 (groin). Lumbar puncture four days later yielded 15 cc. of very slightly yellowish fluid, which cleared on centrifugating. There was a sediment of blood cells but no pellicle. Uncentrifugated fluid showed slightly increased globulin content and nineteen white cells (small lymphocytes) per cubic millimetre. The sugar content was 0.125 per cent. The colloidal gold curve was atypical. There was no growth on culture, and tubercle bacilli were not demonstrable in the sediment. Animal inoculation: 7 cc. of this cerebrospinal fluid was inoculated (peritoneum) into a guinea pig (173) which had already received the bloody fluid. Death occurred twenty-one days after the first inoculation. Postmortem showed definite caseous areas in lymph nodes and viscera, including the lungs. All smears made from suspected areas showed occasional tubercle bacilli.

Lumbar puncture, January 11, 1924: 4 cc. of blood-tinged fluid obtained. This was injected into a second guinea pig (176). At necropsy, eighteen days later, there was evidence of slight but definite tuberculosis. A smear from the spleen was positive for tubercle bacilli. The cause of death was a secondary diplococcus infection.

Lumbar puncture, on April 4, 1924, gave 7 cc. of cerebrospinal fluid. On analysis it was apparently normal and animal inoculation was negative for tuberculosis.

DISCUSSION

There is conclusive evidence that tuberculous meningitis may heal even when the lesions are extensive (2). Unfortunately, there is no successful therapeutic measure. In the above case the factors which favored recovery were: 1. The age of the patient. Under two years of age there is only one case of cure reported and in this there was a fatal recurrence after ten months (3). The greatest number of recorded recoveries have occurred in early adult life. 2. The presence of a raised resistance, evidenced by the slight circumscribed and localized pulmonary lesion. 3. The paucity of tubercle bacilli in the spinal fluid and their subsequent disappearance from it. 4. The absence of meningeal hypertension. At lumbar puncture the fluid never came in a continuous stream. Nevertheless, during the clinical course of the disease the prognosis seemed hopeless; each exacerbation of symptoms looked as if it would terminate the illness. In the later attacks the prognosis was more hopeful, because of the precedent of her overcoming the earlier ones.

SUMMARY

The case here recorded was typically clinical meningitis in its onset and symptomatology. The presence of tubercle bacilli in the spinal fluid, as shown by animal inoculation, proved it to be tuberculous. The unusual features were the number of exacerbations and remissions. It is rather difficult to account for these. They may have been caused by extension of the lesion with new tubercle formation. One of them promptly followed a lumbar puncture, and at the time was considered possibly to represent a tissue reaction to the needle or to the alcohol which adhered to the needle after sterilization. When subsequent exacerbations occurred without such a stimulus the theory was discounted. The disappearance of tubercle bacilli from the spinal fluid suggested circumscription and encapsulation of the lesions. The small number of bacilli and the absence of meningeal hypertension indicated that the lesion was not predominantly of the exudative type, but possibly proliferative in character. This might account for the later recurrences.

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SYPHILIS OF THE LUNG[1]

R. C. KIRKWOOD[2]

The association of syphilis of the lung with pulmonary tuberculosis is reported variously as occurring in from 1 to 11 per cent of all cases admitted to sanatoria for the treatment of tuberculosis (in the experience of the writer in about 3 per cent). Certainly the association occurs frequently enough to make it important, if not imperative, that all patients admitted to institutions for the treatment of pulmonary tuberculosis should have a Wassermann test made on their blood at the time of entrance to the institution.

However, in this paper, it is proposed to consider only pulmonary syphilis as it manifests itself in patients with no tuberculous lesion. During the past seven or eight years the writer has seen a number of cases diagnosed as pulmonary tuberculosis, which proved to be pulmonary syphilis without any demonstrable tuberculous lesion whatever, and we feel that possibly syphilis of the lung occurs more frequently than the literature on the subject suggests.

The history and symptomatology of these cases is much the same as those presented by early or moderately advanced cases of pulmonary tuberculosis, namely, ease of fatigue, indifferent appetite, impaired digestion, nervous instability, a slight rise of temperature during the afternoon or evening, cough, especially at night and in the early morning, with morning sputum, mucoid or mucopurulent in character, often copious and foul smelling, afternoon malaise, shortness of breath, which is more marked than is usual in pulmonary tuberculosis with the same extent of involvement, and in some cases chest pains aggravated by respiratory movement and, more rarely, haemoptysis. The latter may vary from slightly blood-streaked sputum to a small frank haemorrhage. These patients complain of loss of strength, of being always tired, of waking up tired in spite of a long night's sleep, but they do not, as a rule, exhibit the loss of weight one usually finds in cases of pulmonary

[1] Read before the Park County Medical Society, Livingston, Montana, August 7, 1925.

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tuberculosis of like extent of involvement and degree of constitutional reaction.

The physical signs are not constant, but are not infrequently consistent with minimal or moderately advanced pulmonary tuberculosis, or, more often, with chronic bronchitis and bronchiectasis, or both. Inspection as a rule shows little or nothing of importance, the percussion note may be hyperresonant or slightly, if any, impaired except at one or both bases posteriorly, where there is often definite impairment of resonance. Breathing is roughened or indistinct, expiration may be slightly prolonged, in some cases there are localized areas of bronchial or bronchovesicular breathing, and at the bases, sometimes limited to one base, there is tubular breathing. Râles are inconstant, and, when present, will likely be musical or coarse moist ("bubbling") in character. These "bubbling" râles are usually heard only at one or both bases.

Laboratory findings are usually urine negative, but little, if any, change in blood-picture, except perhaps a slight diminution in haemoglobin percentage, sputum repeatedly and constantly negative for tubercle bacilli, at oft-repeated examinations, blood-pressure normal or above normal, blood Wassermann strongly positive in all cases, and, finally, the diagnosis is confirmed by the therapeutic tests, all cases being improved or completely clearing up when given intensive antisyphilitic treatment.

Radiographs show diffuse bronchial thickening (fibrosis) throughout the lungs with dilatation of the bronchi at one or both bases, shadows suggestive of areas of bronchopneumonia in upper as well as lower lobes, and in rare cases very dense, sharply circumscribed shadows usually limited to the lower lobes and interpreted as gummata. Over the bases, one or both, a diffuse, uniform haziness is sometimes seen.

The following illustrative cases have been seen by the author during the past seven or eight years:

Case 1: Mrs. M. W., white, aged 37, married, no children. Denies syphilis in family, but admits father died in a hospital for the insane, the type of his mental disease however being unknown. Past medical history unimportant except for history of one miscarriage at about 6 weeks. Denies syphilitic infection vehemently. Sought treatment for bilateral chest pain, nervousness, loss of strength, dyspnoea on slight exertion, morning cough and expectoration. Physical examination showed a short, obese woman, symmetrical chest, no definite limitation of thoracic movement, impaired resonance at both bases, more marked on right, with normal or slightly hyperresonant note above,

and inconstant musical râles throughout both lungs, with bronchial breathing and coarse mucous râles at the bases. The X-ray showed peribronchial fibrosis, and in the bases several very dense, sharply circumscribed areas, the size of a chestnut to a small egg, and over all of the bases a diffuse haziness. The urine was negative, haemoglobin 70 per cent, and red and white cells normal in number and morphology. The blood-pressure was 135 systolic and 90 diastolic. Repeated sputum examinations were negative for tubercle bacilli. The blood Wassermann was four plus. After a course of neosalvarsan with mercury and intravenous sodium iodide, and with rest, general hygienic regimen and tonic medication, the patient improved in every way. The Wassermann became negative, and when discharged the patient reported she felt better than she had for years. No post-treatment X-ray was taken in this case.

Case 2: M. C., white, aged 32, single, graduate nurse. Negative family and past medical history. Denied syphilis. Referred with a diagnosis of pulmonary tuberculosis. Complained of marked but gradual loss of strength, with practically no loss of weight; cough, severe at night and early in the morning, with considerable morning expectoration, mucopurulent in character and at times very definitely blood-streaked; marked dyspnoea on slight exertion; poor appetite and impaired digestion, manifested by the sensation of being bloated after eating; and extreme nervousness and insomnia. Physical examination showed signs of a diffuse bronchitis throughout both lungs (roughened breathing and inconstant musical râles), with areas of bronchial breathing and coarse râles in midlung, bilaterally and at bases, with increased transmission of whisper over these areas. The X-ray showed diffuse peribronchial fibrosis, with small areas of increased density, less marked and less sharply defined than the dense areas seen in case 1, with increased hilar shadows and dilatation of bronchi to bases. The sputum was constantly negative for tubercle bacilli at many examinations over a period of months. The blood-pressure was systolic 130, diastolic 85. The blood Wassermann was strongly positive at several examinations before institution of specific treatment. Under rest, general hygienic regimen, postural drainage of bronchiectasis and intensive antisyphilitic treatment the patient improved rapidly and regained her strength; appetite and digestion improved; she became much less nervous and was able to sleep well and awake refreshed; and when discharged was well except for the persistence of a slight cough and morning expectoration. The X-ray when discharged showed only fine peribronchial fibrosis with bronchiectasis to bases, the latter less marked than when first examined. This patient when seen more than a year later was working hard as head nurse of a hospital in New Mexico, and said she felt perfectly well.

Case 3: J. H., white, single, aged 43, chief engineer of a steam-vessel plying in tropical waters. Family history negative. Had always been rugged and healthy. Admitted syphilis some 15 years previously, which apparently had been inadequately treated. Lately had noted that he tired more easily than usual; became short of breath after slight exertion; had a cough, most severe just after going to bed and in the early morning hours, with early morning expectoration of copious amounts of foul-smelling mucoid sputum, and chest pain, increased by deep breathing. There was little, if any, loss of weight, the patient being well-muscled, large-boned, and weighing nearly 200 pounds, though not obese. The physical signs were roughened breathing and inconstant musical râles throughout both lungs, with bronchial breathing and "bubbling" râles at right base. The X-ray showed diffuse peribronchial fibrosis, with slight general haziness over right base and dilatation of bronchi to right base. The blood pressure was systolic 160, diastolic 100. The urine was negative at several examinations. The sputum was negative at repeated examination over a prolonged period. The blood Wassermann was four plus. Under rest, postural drainage of bronchiectatic cavities and specific treatment (salvarsan, mercury and sodium iodide) the blood Wassermann became negative. The patient regained his strength; signs and symptoms improved or cleared up entirely; and when discharged the patient reported feeling better than he had for a long time. The X-ray after treatment showed that the general haziness had cleared up and that the bronchiectatic dilatations were smaller than before treatment. The patient returned to his maritime occupation, and when last seen a year or more later was working hard, except for a slight morning cough and expectoration, which he attributed to "catarrh" which he said he had had "for years."

Case 4: G. M., white, widower, aged 46, broker. Family history negative. Past medical history unimportant, except for syphilis some 12 years before, for which treatment was possibly inadequate. The patient complained of being always tired, not rested by hours of sound sleep, very nervous, irritable, insomnia, no loss of weight, a morning cough with considerable "watery" expectoration of foul odor, marked dyspnoea on slight exercise, poor appetite and impaired digestion. Physical examination showed a well-nourished, well-muscled adult, tongue coated and breath foul. The chest signs were harsh breathing and inconstant musical râles throughout both lungs, with bronchial breathing and "bubbling" râles at left base. The X-ray showed diffuse peribronchial fibrosis, with dilated bronchi to left base. The urine was negative. The blood-pressure was 155 systolic and 95 diastolic. The sputum was repeatedly negative for tubercle bacilli. The blood Wassermann was strongly positive. Under rest, general hygienic treatment, tonic medication and spe-

cific treatment for the syphilis, the Wassermann became negative, and the patient regained his sense of general well-being; his strength returned; and after his discharge he returned to his work as a stock-broker, reporting that he was able to work long hours without undue fatigue. Only a slight cough and morning expectoration remained when he was last seen, and these were of no importance.

Though not specifically mentioned, all of these cases, when first seen, had a daily afternoon or evening rise of temperature of from 99.2° to 100°, which promptly cleared up under treatment, none of the cases having any rise of temperature at the time of discharge or when seen later.

It is of interest that when case 1 presented herself for treatment her husband was then, and had been for months, in the hospital with an ununited fracture of the femur. The wife's positive Wassermann suggested a possible explanation of the nonunion of the husband's fracture, and a Wassermann test was made on him and was reported four plus. Specific treatment was instituted and satisfactory union of the fracture resulted in a short time.

CONCLUSIONS

1. Syphilis of the lung is apparently not so rare as we have been led to believe.

2. Pathologically, three types of lesion present themselves: (1) diffuse peribronchial fibrosis, with bronchiectasis at one or both bases (the so called "fibrous induration"); (2) gummata, single or multiple; and (3) consolidation and catarrh, the so called "bronchopneumonic" type. In acquired syphilis, at least, the diffuse fibroid induration type is most common. In congenital syphilis gummata are possibly most frequently seen.

3. While syphilis of the lung simulates pulmonary tuberculosis to such an extent that careful, and often repeated examinations, and painstaking histories and clinical study of the case, are necessary to clear up the diagnosis, the differentiation can be made.

4. The constant absence of tubercle bacilli from the sputum at oft-repeated examinations and the presence of a positive blood Wassermann suggests the diagnosis.

5. And, finally, the diagnosis can only be proved by complete disappearance of symptoms and physical signs, or their marked improvement, following the administration of antisyphilitic treatment.

THE TREATMENT OF BILATERAL LUNG SUPPURATION[1,2]

J. J. SINGER AND EVARTS A. GRAHAM

In the past few years considerable interest has been manifested by physicians, as well as surgeons, in suppurative conditions of the lung. Under this head are the following pathological conditions: abscesses, acute and chronic; bronchiectasis; purulent bronchitis; empyemata that are draining through bronchi; and tumors of the lung which have broken down. It is often very difficult to differentiate these conditions and it has become more or less the general custom to classify all of them as suppuration of the lung.

In previous papers we have discussed bronchiectasis (1) and abscesses of the lung (2) as such. It is the purpose of this paper to discuss bilateral suppuration and its treatment with a report of five cases.

Before the World War it was unusual for surgeons to undertake operations even on one lung because of the great dangers incident to this class of cases. But time has taught us that the operations on the lung are relatively safe with the newer methods. Bilateral lung cases are still considered by many as inoperable. However, we have found it possible to treat many such cases with excellent results and often with very simple methods.

Before attempting the more complicated procedures, it has been our custom to use the simplest methods for drainage of lung suppuration. The first method is postural drainage three or four times a day on a specially constructed tip top table as illustrated. This table is constructed on the "see-saw" principle, allowing the patient to remain in the dependent position for only a minute or two. Occasionally pus will drain at once; at other times, the patient will cough up a considerable amount shortly after the drainage. The relief of the persistent cough following this simple procedure is often very striking. With this frequent drainage the purulent condition of the lung is often lessened

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in a short time, and at times permanent cures follow. The method must be used carefully, however, as occasionally hemorrhages have resulted probably from increased hyperemia of the granulation tissue always present in these suppuration cases.

If, however, cases do not respond in four weeks, bronchoscopy[3] is used; first, to establish the presence or absence of a foreign body in a bronchus; second, to dilate bronchi; and, third, to aspirate pus. Occasionally antiseptic oils are injected into abscesses. It has been noted that no great amount of pus is really aspirated from the suppurative areas, but the strain relative to the cough which follows the dilatation of the tubes and the removal of granulation tissue makes better drainage through the natural bronchial drainage passage. We also use postural drainage in addition to bronchoscopic drainage. This tends to keep the bronchi open.

The next step is the use of pneumothorax compression. There has been considerable discussion as to its value in this condition, especially when it is bilateral. While it is true that adhesions are often present in suppurative lung conditions, one can never tell from physical signs or the roentgen-ray examination whether they are present or not. It is only by the introduction of a needle connected with a manometer that their presence can be detected.

We have had many surprises during this procedure; in cases in which one would expect to find adhesions everywhere large pleural spaces were found, and the introduction of air was a simple procedure. The results we have obtained in several cases are almost miraculous.

In bilateral cases of bronchiectasis, it is necessary to find, by careful study and analysis of signs and roentgen ray, which lung is performing most of the work. This is not difficult, as one can see by physical signs and in the fluoroscopic examination which lung is expanding the most. When one considers that a bronchiectatic lobe is usually also atelectatic and therefore functionless, the compression of that side should not materially change the vital capacity.

The proper interpretation of the roentgen plate is also very important. It is often noted that the so called "good lung" is thickly marked and spotted, and, if that criterion alone were used, we would hardly consider these cases suitable for collapse therapy. We have found that collapse of the one side allows for greater aeration of the other, with

[3] If there is any suspicion of a foreign body, bronchoscopy is of course performed at once.

marked improvement of the less affected side. The explanation of this phenomenon may be that the infection from the worse side is prevented from constantly reinfecting the contralateral lung. We have used postural drainage as described in conjunction with this compression. Good results are noted usually after two or three compressions, which are generally given every four or five days. If symptoms are not ameliorated after two or three months, we find that this type of case is then subjected to surgical treatment. Spirochetal infections of the lung are treated by salvarsan.

The surgical methods used in lung suppuration are as follows: (1) thoracoscopy, (2) surgical pneumothorax, (3) partial thoracoplasty, (4) complete thoracoplasty, and (5) cautery pneumonectomy.

(1) *Thoracoscopy* is done occasionally when it is found that tumors or abscesses are the probable diagnoses. First, a pneumothorax is performed by the usual method; then, under twilight sleep and local anesthesia, the thoracoscope devised by one of us (3) is inserted in an intercostal space. It is often possible by this means to see whether the mass is connected with pleura or is a part of the lung tissue itself. Sections of tissue may also be removed.

(2) *Surgical pneumothorax* is used when it is decided that an exploratory operation is necessary. If few or no adhesions are present, the pleural space is allowed to fill with air, and the pleura and chest tissue are closed. It has been found that the purulent areas are completely compressed and may become normal in a short time by this relatively simple procedure. Occasionally it has been found necessary to keep up this air compression by artificial pneumothorax every week or two. If the lung shows evidence of consolidation without abscess formation, we often sew this portion to the chest-wall so that adhesions at this site will make cauterization, if necessary, simpler and safer. The value of this procedure is due to compression of the lung and the fact that the cough is greatly lessened. It is not possible to cough unless the air is breathed or aspirated into the depths of the lung. The vicious circle produced by constant cough is thus prevented, and absorption of the infected material results. If this treatment is combined with postural drainage even greater and quicker results follow.

(3) *Partial thoracoplasty*, especially in lower-lobe involvement, has been done, especially in cases in which it is not advisable to do a great deal surgically. A pad fitted to a bandage is placed over the operative area to make compression more affective. At best this is only palliative.

(4) *Complete thoracoplasty* is recommended by some in unilateral cases, but we have not used this method. For example, Hedblom (5) has had some excellent results from graded thoracoplasty in cases of unilateral bronchiectasis. One case in our series had this operation performed elsewhere with complete surgical healing. The compression of the lung was not sufficient to produce fibrosis. In fact, many cases subjected to thoracoplasty are not improved. In comparing pneumothorax compression with thoracoplasty compression we note that much greater benefits result from pneumothorax, but the hard fibrous tissue around cavities often prevents the desired results.

(5) *Cautery pneumonectomy* (4). This procedure represents all surgical requirements for infected tissue. If the lung is not found adherent at the time of operation, it is sewed to the chest-wall after a rather large exposure of the pleura. Portions of three or four ribs are removed over the site of the greatest involvement. Five to eight days later, by means of the actual cautery, a large area of lung is burned, but at first no attempt is made to burn very deeply. One may find many foci of pus. Later a gauze pack is inserted into the crater formed by this cauterization. This prevents hemorrhage and also acts as a compression on the pulmonary tissue. From six to eight days are required for the slough to drop off. Later we have a deep wide opening into pulmonary tissue with the bronchi acting as drainage-tubes. To facilitate evacuation of pus we use an aspirator-bronchial tube, thus preventing a great deal of strain, as patients must otherwise force this pus by coughing.

In addition to these procedures we use heliotherapy, both natural and artificial, as a general treatment and also as a local treatment. We have found excellent results following these methods and feel that by close, constant and detailed attention many of our most miserable cases can be benefited.

Case 1: E. B., 6 years. *C. C.:* Lung abscess. *F. H.:* Mother and father living and well. There are two children, the patient and a girl of five years. No children dead. No history of tuberculosis or "lung trouble." *P. H.:* Normal delivery at term, weighing 7 pounds. Breast-fed for two weeks, then put on bottle feedings, using Eagle Brand milk from two weeks to one year of age. Development normal as to teething, talking and walking. Had no summer complaint, no colds, no sore throat.

P. I.: Patient's breathing was so noisy in the winter of 1920 and 1921 that in that spring his adenoids were removed. During the fall of 1921

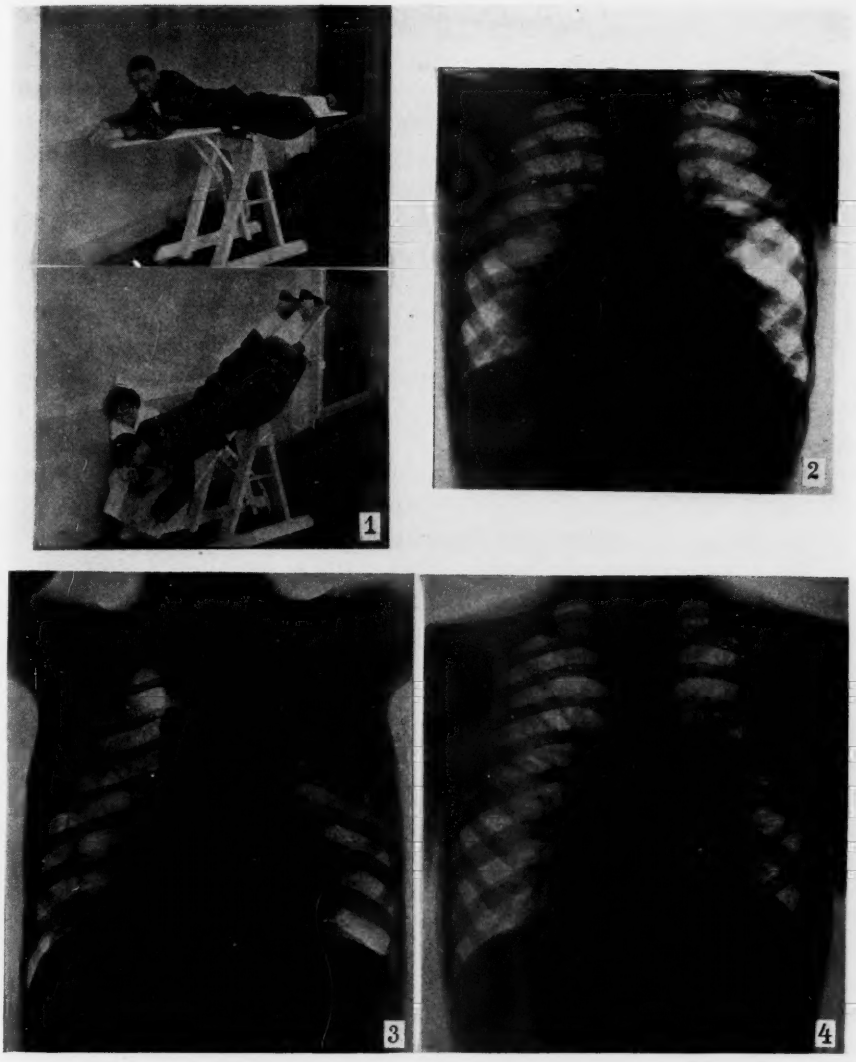

FIG. 1. METHOD USED FOR POSTURAL DRAINAGE

Collection of sputum in sterile petri dishes. The angle of the table can be regulated according to necessity.

FIG. 2. E. B., FEBRUARY 7, 1924 (AET. 7 YEARS)

Bilateral bronchiectasis more extensive on the right than on the left. Condition present for four years. Physical signs showed the left side more compensatory.

FIG. 3. E. B., FEBRUARY 21, 1924

Pneumothorax instituted on the right side, showing marked improvement in the densities.

FIG. 4. E. B., JANUARY 31, 1925

Showing the clearing up of the bronchiectatic areas in both lungs following the use of pneumothorax in the right chest alone. It is interesting to note that this patient has had chronic sinusitis, and even greater improvement resulted following its surgical treatment.

periods of fever and cough and purulent sputum began and have continued without much change since. During the intervals between attacks he is well, has no fever and no cough, and a good appetite. Then over night he becomes ill with fever, cough with much sputum, dyspnea and orthopnea. Attacks last from two weeks to two months. He has had much treatment since 1921, including osteopathy, Christian Science, Abrams's treatment and hydrotherapy.

In the spring of 1923 he had measles and pneumonia and was sick until June, 1923. Had pertussis in November.

Has been taking drainage exercises before each meal since onset, to drain the cavity in his lung. The mother says that between attacks there is no drainage, but over night another attack may come on and he will bring up large quantities of sputum. Present flare up began March 8, 1924.

P. E.: Temperature 39.2°C., breathing rapidly, but with no apparent effort. Coughs at intervals, and brings up considerable amount of yellow pus which has little odor. The physical signs (March 11, 1924) show diminished breathing at the right base, and dulness. The mid-portion of right lung shows increased whispered voice. This was more marked three days ago. In the left lung the breath-sounds are harsh, and coarse râles are heard, but this breathing is compensatory. X-ray, however, shows thickening of the markings especially from the hilum down. X-ray, physical examination and history make a diagnosis of bilateral bronchiectasis and an acute pneumonitis, worse on the right side.

The treatment (March 13, 1924) consisted of artificial pneumothorax on the right side which was done five times on the dates as follows: March 13, 275 cc. of air; March 15, 275 cc.; March 19, 300 cc.; March 24, 350 cc.; March 31, 1924, 350 cc.; all on the right side and with good collapse. The patient had no reaction from any pneumothorax. Always felt better afterward and coughed up large quantities of sputum following the treatment. A small amount of fluid formed, but was sterile.

Sputum, blood and urine negative. Temperature gradually dropped. Sputum ranged from 180 to 240 cc. daily while in hospital, but was much decreased when patient was discharged from hospital. Patient discharged from hospital April 2, 1924, much improved to return for observation.

From this time until December 1, 1924, he received fifteen right-sided pneumothoraces with no unusual reactions. During this time he had several mild nasal infections and an abscess on the right buttock and broke his right arm.

On December 13, 1924, a slight increase in induration at the left hilum was noticed, and the patient was bringing up a little more pus. Sinuses treated by Doctor Sluder. Lungs began to clear up. Tonsillectomy January 4, 1925.

Lungs showed marked clearing. Vital capacity 800 cc. Weight increased from 59 to 66 pounds. Less coughing and patient feels much better.

Case 2: T. R., was sent into Barnes Hospital by Doctor Bicknell of East Chicago, Indiana, with a chief complaint of cough which became more productive after operation on November 2, 1923, for cholecystitis and appendicitis. The family history and past history are negative.

P. I.: Started with a "cold" in September, 1923. At that time patient had a cough and a sense of tightness in the neck above the suprasternal notch. He went to several doctors, one of whom finally operated on him, November 2, 1923, removing his gall-bladder and appendix, although no history of symptoms pointing to these can be obtained. One week after operation cough became worse and more productive. Sputum occasionally blood-streaked. The operative site in the abdomen did not heal promptly, discharging a foul-smelling pus. On about the first of January, 1924, patient went to Doctor Bicknell who "stood him on his head" and obtained about two ounces of very foul-smelling pus.

P. E.: Head: Two scars on face due to fall. Small "cryptic" tonsils. *Chest:* Breathing mostly abdominal. Percussion note diminished from 4th rib down on right, posteriorly. Breath-sounds somewhat harsh over right base, anteriorly. From left of scapula down, breath-sounds are of tubular quality. Whispered sound increased. Fluoroscopically there are many small cavities in the right middle and lower lobes. Suspicious fluid level in one of these cavities with air above. Much increase of hilum shadow. Thickening of lung markings, especially those toward the apices, which are beaded. *Abdomen:* Shows marked postoperative hernia, with bulging on coughing. Small discharging sinus. *Sputum:* Greenish yellow, tenacious, many polymorphonuclears, no tubercle bacilli. *Urine:* Negative. Red blood cells, 4,310,000; hemoglobin, 65 per cent; and leucocytes, 11,000.

On January 19, 1924, using novocaine (0.5 per cent), a portion of the ninth and tenth ribs were removed subperiosteally about the right scapular line. Lung adherent to pleura. Small amount of thick pus aspirated. Large cup-shaped depression burned out with cautery. Following operation patient continued to bring up 30 to 50 cc. of sputum, with drainage also from operative wound. This continued for about three weeks. Then the wound in the chest stopped draining, closing with granulations. The sputum increased.

On February 15, 1924, with nitrous oxide-oxygen anesthesia, the former incision was opened up widely and two more ribs taken out. Intercostal bundles also removed. Skin sutured back from wound to prevent too rapid closure. A piece of the pleura removed showed chronic pleuritis. Following this operation patient improved somewhat, and gained weight. Vital capacity 1100 cc. Leucocytes 12,500. Cough continued. Sputum examination negative for tubercle bacilli. The wound continued to drain until the sinus was practically closed on April 28, 1924. Then the patient began having much pain in the right chest, causing loss of sleep. Fluoroscopy showed dense irregular shadow throughout the right base.

On May 5, 1924, with nitrous oxide-oxygen anesthesia, an incision was made around the draining sinus down through scar tissues to the surface of the lung. This sinus was found to communicate directly with the lung, and after slight coughing a little pus was expelled through a small opening in the sinus. The cautery was plunged into the sinus, and an abscess cavity was entered with a capacity of about 15 cc. This contained thick, inspissated and foul-smelling pus. There was also a small sequestrum in it (rib?). The roof of the cavity was burned off with the cautery to make a shallow saucer-like depression. No hemorrhage. No sutures taken. Following the operation the patient improved somewhat. Cough was considerably less. Less sputum. Leucocytes fell to 5,650. This improvement continued for about six weeks. Heliotherapy was employed.

On July 3, 1924, patient coughed up a cupful of blood. Cough became worse, but sputum diminished. Leucocytes 13,800. Drainage was not satisfactory. On July 16, 1924, the old incision was enlarged by extending it forward. With the cautery a large saucer-shaped depression was burned out of the lung, beginning at the site of the old drainage and extending around forward. The high position of the diaphragm made access to the lung somewhat difficult. No sutures. There was not a large amount of pus encountered, but several pus pockets were burned out. Heliotherapy was continued. Patient continued to cough but slept well. His vital capacity was 1000 to 1200 cc. Combined aspiration and heliotherapy tried. Sputum repeatedly negative for tubercle bacilli.

Vital capacity rose to 1,650 cc. on October 6, 1924. The lung wound healed slowly and the bronchial fistula began to close.

On October 21, 1924 patient's ventral hernia was repaired, using fascia lata sutures.

On December 10 numerous spirochetes were found in a culture from the wound, also fusiform bacilli. Patient was put on salvarsan therapy for his lung spirochetosis. He was discharged to the out-patient department with his lung wound still draining slightly. His cough had practically disappeared.

From January 8 until approximately January 29, 1925, patient was free from cough. Then it came back, hacking in character. Later it became productive and his breath got very foul. In the out-patient department patient received five injections of salvarsan of 0.5 grams each.

On March 3, 1925, patient came back to Barnes Hospital, complaining of pain in his left side and shortness of breath. Examination of the chest at that time showed numerous râles in the left hilum and a slight increase in breath-sounds at this area. Fluoroscopy showed an area of increased density in the left hilum. There was good movement of the left diaphragm and a clear angle. It seemed that the process in the right lung, on which the operations had been done, had extended over to the left hilum.

The area rapidly developed into an abscess, with fluid level which could be seen on March 9, 1925. The abscess was more anterior than posterior. Postural drainage was carried out from March 9 until April 2. Pneumothorax was lightly considered on account of the extensive involvement of the right lung.

The abscess rapidly extended until there was danger of it rupturing into the pleura on April 1. It was then decided to perform an operation in two stages for surgical drainage. The vital capacity of the patient was only 800–900 cc., very little more than his tidal necessity. If a surgical pneumothorax were produced it would probably be fatal.

April 2 an incision parallel to the third rib on the left side from the anterior axillary line almost to the sternum was made. The third rib was removed subperiosteally to be sure to avoid a pneumothorax. Sutures were taken through the lung and the chest-wall without opening the pleura first, to be sure of the presence or the absence of adhesions. A needle was inserted through the sutured portion and about 10 cc. of very putrefactive and bloody pus was removed. The wound was packed with iodoform gauze to promote adhesions. The ends of the ribs were packed with bone wax and the nerves injected with alcohol.

April 4 the second stage was done. With the cautery the abscess was opened, and about 50 cc. of pus evacuated. It was necessary to cut the pectoral muscle to get proper drainage.

Patient did poorly for about two weeks. He had several hemorrhages from his operative site. One of these on April 11 was very severe, patient coughing up blood and losing quite a bit from the wound. He was typed (group II), and transfused with 500 cc. of blood. Patient rallied wonderfully, and on April 19 his temperature was back to normal. He improved rapidly and remarkably. His sputum became less, finally getting down so that the entire twenty-four hour amount did not cover the bottom of cup. He looked very much better. Temperature and pulse continued normal.

On April 27 patient had "no odor at all" (previous to his last operation all the patients in the ward were complaining of it). He is up and about the ward and sleeps out in the open air.

X-ray on April 22 showed a remarkable clearing of the lungs. At present the wound in the left chest is entirely healed but on the right side he has a bronchial fistula.

Case 3: B. P., male, 25 years old, white, entered Barnes Hospital May 20, 1924, and was discharged June 3, 1924. Condition improved, no operations.

C. C.: Persistent cough, large quantities of foul sputum. *F. H.* unessential, and *P. H.* of usual childhood diseases. Double pneumonia when nine years old, followed by an unproductive cough which has continued to onset of present illness five years ago. Otherwise negative.

P. I.: Patient had double pneumonia at nine years of age, very severe, and was in bed for year following, because of slow recovery of strength. Had a dry unproductive cough which continued until he was 20 years of age. On December 22, 1920, patient was exposed to elements, and contracted influenza. In bed two weeks but recovered rapidly; however, he had a residual cough with quite profuse inoffensive sputum. Patient has never been free from this, and condition was much aggravated during winter months, or when lying down or stooping over. In the morning he wakes feeling as though throat were full, and after coughing and emptying throat and lungs he is not bothered much for the rest of the day. Sputum has become thick, greenish and foul-smelling, and patient brings up large amounts at a time, coughing very little between times. He has been able to work every day, feels well and has lost no weight.

P. E.: Positive findings only: *Chest:* Left upper more prominent than right. Limited expansion on left. Percussion note impaired over left anterior and posterior upper, dull at left base. Tactile fremitus and whispered and spoken voice much increased on left, anterior and posterior. Many coarse bubbling râles heard on left side. Suggestion of splash in left chest when heart beats (300 cc. of air injected into chest this morning). Right chest hyperresonant. No râles. Heart apex felt in midaxillary line, 6th interspace.

May 22, 1924, out-patient department history, additional data only. No pain in chest except just before coughing. Slight amount of blood in sputum in last few weeks. Occasionally has night-sweats. Sputum more profuse in morning. Patient still working but feels quite weak. Cannot lie on right side without coughing.

Course in hospital: May 20, 1924: Postural drainage produced large quantity of greenish, foul sputum. To be repeated two to three times daily. May 20 (Dr. Singer): Physical signs and X-ray show bilateral bronchiectasis with abscess on left side; suggest pneumothorax, 300 cc. air introduced into left chest cavity. No adhesions encountered. May 23 (Dr. Singer): Pneumothorax still present. May 24 (Dr. Singer): Good collapse from apex to diaphragm, 450 cc. air injected. May 26 (Dr. Singer): Patient shows a good collapse of left lung after 48 hours, looks better and brings up less sputum. Mediastinum not pushed over. Minute amount of fluid in costal angle. May 29 (Dr. Singer): Pneumothorax, good result. Evidently adhesions were loosened. June 2 (Dr. Singer): Pneumothorax, good, almost complete collapse. Still coughs, but very little. Discharged June 3. Physical findings indicated bronchiectasis or lung abscess. Fluoroscopy showed both to be present; treated with postural drainage and pneumothorax. Condition improved.

Laboratory: Urine negative. *Blood:* Leucocytes, 8, 250; red cells, 4,880,000; hemoglobin, 90; Wassermann negative. Temperature, 36° to 37.6°C. Sputum consistently negative for tubercle bacilli.

Subsequent course: June 9: Pneumothorax, 450 cc. June 17: Pneumothorax, 450 cc. Less cough and sputum. June 29: Pneumothorax, 475 cc.

To date patient has been given pneumothorax about every two weeks, with constant improvement in feeling, increase in weight, less cough and very little sputum of an inoffensive character. Only on one occasion was there any reaction to the pneumothorax. At this reaction patient complained of tight feeling in chest. Pupils were unequal and patient was emotionally upset. Never has there been more than a slight amount of fluid in costal angle. Patient is back at work as usual with none of the original weakness.

Case 4: H. P., age 19, female, white, with *C. C.* (October 27, 1924) of chronic cough with abundant sputum. *F. H.:* Unimportant, and *P. H.* of pertussis at three years of age. Has had a cough ever since, and has brought up small amounts of "phlegm." Measles when an infant. Pneumonia at 3, 12, 17 and 19 years of age. No complications with pneumonia until last attack in April, 1924. Since this time the cough has persisted. Sore throat often during childhood. Influenza in 1918. With last attack of pneumonia patient had painful and swollen knee-joints, which lasted about five days. *Menstrual history:* Onset at 14, regular. Has had much the last few periods. Last period October 4.

Onset of *P. I.* last April (1924), when patient had an attack of pneumonia, complicated by painful and swollen knees. Was sick for two weeks. The cough, of which patient complains, began when the pneumonia was resolving, and has persisted. Sputum was clear for two weeks, and then became thick and yellow; lately it is greenish. Patient has a good appetite at times and at other times none at all. Has lost 20 pounds in six months. Occasionally has an afternoon fever and night-sweats. At times, when cough is worse, she has pains, mostly in the lower chest on the left side. Lately, pain has been on both sides, anterior and posterior. Some dyspnea now; does not breathe deeply because it produces cough; sputum is easily produced, varying from ½–2–3 cups daily. Stooping causes an excessive flow of sputum. Sleeps propped up in bed with three pillows, because in this position she coughs less. Says sputum is greenish-yellow, with very foul odor, no blood. Red cells, 4,890,000; leucocytes, 4,250; hemoglobin, 86 per cent; culture negative. Polynuclears, 71 per cent; lymphocytes, 22 per cent; large mononuclears and transitionals, 3 per cent; eosinophiles, 4 per cent. Temperature 104° upon admission, with gradual decrease. Wassermann negative. Blood pressure, 105/70 rather constantly. Urine negative. Sputum negative for acid-fast bacilli.

P. E.: Patient lying propped up in bed with apparently much discomfort; frequent coughing with production of greenish-yellow sputum. Cough more severe when patient is lying flat in bed. Skin warm, moist and elastic. Palms and soles especially moist. Face very pale. Nail-beds and visible mucous

membranes pale. Weight, 99 or 100 pounds. Tonsils moderately enlarged and ragged. Small palpable lymph nodes in both anterior triangles of neck. Thyroid not enlarged.

Chest: (Dr. Graham and Dr. Singer): Supraclavicular fossa prominent. Respiration 30, shallow with frequent cough. *Left apex*, anterior and posterior normal; *right apex*, anterior and posterior normal. Physical signs and X-ray show involvement in left base, posteriorly. There is a suspicious involvement of right lower, but there is compensatory emphysema. Artificial pneumothorax was advised.

First pneumothorax October 28; vital capacity 1000 cc.; 300 cc. air introduced; good collapse of left lung. Some adhesions at base of lung to diaphragm.

October 30 (Dr. Singer): Bronchovesicular breathing still present but to less degree in left lower. Râles have largely disappeared in this area. Patient says that she feels much better; less cough and sputum.

October 31 (Dr. Singer): Second pneumothorax done; 500 cc. air introduced; no reaction.

November 2: Cough and sputum much less. Patient feels much better.

November 5 (Dr. Singer): Third pneumothorax started; 250 cc. air introduced, and patient had sudden coughing attack with production of much pus and dyspnea; it lasted twenty minutes. Fluoroscopic examination showed that lung was completely collapsed with only 250 cc. air. Patient had a spontaneous collapse in addition to artificial one.

November 11: Patient continues doing well. Temperature 37° most of time. Cough and sputum less.

November 12 (Dr. Singer): Fluoroscopic examination shows lung still collapsed but not completely so. Fourth pneumothorax, left; 500 cc. air introduced; excellent collapse.

November 20 (Dr. Singer): Patient allowed up in wheel-chair. Fifth pneumothorax, left; 400 cc. air injected, left lung collapsed; right one compressed.

November 27: Sixth pneumothorax, left; 350 cc. air injected, patient had severe pain in right side all night.

December 2 (Dr. Faulkner): Postural drainage, 30 to 40 cc. yellow, foul, thick sputum.

December 3: Postural drainage, 30 cc. December 4: Postural drainage 30 to 40 cc. December 5: Postural drainage to continue daily, patient feels much better.

December 6: Seventh pneumothorax, left, 500 cc. air.

December 13: Eighth pneumothorax, 450 cc. air, no reaction, severe nausea and dyspnea at night.

December 16 (Dr. Graham, Dr. Singer and Dr. Barr): Suggest withdrawal

of air from left chest and surgery later; 400 cc. air withdrawn. Lung came out to chest-wall, with mediastinal pneumothorax to apex and peripheral at base.

January 2, 1925 (Dr. Singer): Left base same as upon admission, patient shows no improvement since reëxpansion of left lung. (Drs. Graham, Singer and Barr): Decided to operate on left side.

January 6: Transferred to Surgery.

January 13: Under gas anesthesia 3 ribs resected on left side. (First stage thoracostomy done). Pleura ruptured spontaneously. Lung sutured to pleura. Left operation room in good condition. Seemed all right for 5 or 6 hours, then became cyanotic and developed attacks of coughing. Was given oxygen and respiratory and cardiac stimulants, but died two days following operation.

Case 5: R. T. white, female, aged 21. First seen in out-patient department April 28, 1925. In Koch Hospital (1922) 2½ years before entry to Barnes Hospital, October 11, 1920. Then in Convalescent Home and Jewish Hospital before second entry to Barnes Hospital on November 24, 1924. Patient has had cough with sputum since pertussis at age of five years. In City Hospital at numerous intervals for short periods. In Night and Day Camp for 10 months.

C. C.: Cough, weakness, loss of weight, fever and anorexia. *F. H.:* Unessential. *P. H.:* Pertussis at five years followed by unproductive cough. Pneumonia in 1918, followed by productive cough with inoffensive cough which has continued to date. There have been periods, however, when very large quantities of foul sputum have been raised. Patient had second tonsillectomy in 1920, after which time she has had a continuous fever. At ten years of age had persistent eczema of scalp and axilla.

P. I.: Condition dates back to onset of cough at five years of age, with gradual development of cough, with addition of foul sputum in large quantities (6 years ago) and fever since 1920. Gradual loss of weight, weakness and anorexia, and development of a pain over precordial area.

P. E.: Chest symmetrical, expansion poor, no lag. Percussion note hyperresonant in lower left, posteriorly, impaired right base, posteriorly and medially. Breath-sounds distant. High pitched râles throughout. Voice sounds accentuated along right paravertebral border.

Additional data (Dr. Singer) November 25: Diaphragm sluggish on right. Breath-sounds diminished, both bases. X-ray shows bilateral bronchiectasis. Considerable thickening in lower left lobe behind heart. Dense thickening in lung at right base.

Course in hospital (last admission only): November 26: X-ray shows involvement of left upper apex, not present in plates of four years ago. Tuberculosis suspected. Many squeaks at left apex, anteriorly and posteriorly. Left base compensatory.

January 7, 1925: Pneumothorax three years ago accomplished nothing because of adhesions at right base, so surgical pneumothorax with freeing of adhesions considered. *Operation:* Resection, 7th, 8th and 9th ribs in axillary region. Lung not adherent to pleura. Thickened greatly at base and adherent to diaphragm. Lung sutured to parietal pleura.

January 17: *Operation:* Cautery pneumonectomy right lower lobe. Small amount pus from lung; 10 cc. from small anterior empyema cavity (from previous operation). Small amount of fluid developed at right base. Aspirated on numerous occasions, and only once was small amount of pus obtained. Pneumothorax exists, with lung compressed in mediastinum and fluid at right base. Course somewhat stormy with numerous rises in temperature. Two small hemorrhages from lung.

February 25: Blood transfusion, 500 cc.

April 4: Fluoro-lung fields clearer than previously noted. No fluid level. Slight pneumothorax still present.

April 25: Patient feels much better. Temperature approaches normal, with occasional elevation. Sputum unchanged in character. Slight decrease in volume, less offensive.

Wassermann negative; sputum consistently negative for tubercle bacilli. Red cells, 4,250,000 at entry; 3,425,000 minimum and 4,090,000 at present. Leucocytes, 15,000 at entry; 49,400 maximum and 12,600 at present. Hemoglobin, 90 per cent at entry and 75 per cent at present. Urine has been negative except for occasional very faint trace of albumin. Vital capacity, 2,000 cc. on November 29; 1,650 cc. on December 29.

May 24, 1925: Condition of patient improved, although she brings up moderate amount of purulent sputum. The final result of course is not known, but case shows the feasibility of operation on the worse side. Patient states she is more comfortable.

SUMMARY

In view of the prevalent ideas that bilateral bronchiectasis cases are more or less doomed to constant invalidism, we felt that this class of cases was suitable for study. It has been shown that with persistent care many may be much improved and even may become entirely well.

Case 1 shows that even in children pneumothorax can be done with safety, and the reëxpansion of the lung may be complete with cessation of the treatment. It also shows that the opposite lung is benefited providing the bronchiectasis is not too far advanced.

Case 3 shows that pneumothorax with postural drainage can produce healing even in long standing cases. The plate taken before operation

would indicate extensive adhesions, but the manometer with introduction of air by pneumothorax showed a completely open pleural space.

Case 4 shows that careful roentgen-ray study and physical signs make it possible to find out which lung is the more involved. Death in this case was probably due to surgical shock. No cauterization was done.

Case 5 is still in progress, but good results are expected.

In case 2 almost every conceivable involvement of pulmonary tissue was encountered. The history and physical signs and roentgen-ray studies, followed by many operations, show conclusively that bilateral operations are feasible if the process demands it. The great amount of study and detailed attention have brought its reward.

CONCLUSIONS

1. Careful analysis of physical signs and X-ray makes it possible to determine which lung should be treated or operated upon without affecting the vital capacity.
2. Very simple methods often bring startling results.
3. Two of these five patients apparently recovered by the use of pneumothorax alone.
4. It is possible to operate on both lungs of patients with bilateral suppuration.

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PNEUMOPERITONEUM IN THE TREATMENT OF TUBERCULOUS PERITONITIS[1]

A Further Report

OSCAR MONROE GILBERT[2]

In January, 1924, I reported two cases of tuberculous peritonitis with serous effusion (1) treated by aspiration of the fluid and the introduction of filtered air. These have remained well and are working, one after five years and the other for nearly two years.

I now wish to report two additional cases treated by this method.

Case 3: G. M., negro, age 20, was seen May 14, 1924. He had had pleurisy on the left side, and had been very much run down two years before, but had partially regained his health and was fairly well, being able to do light work until two weeks before admission, when he began to be weak, had fever and malaise, and then noticed that his abdomen was becoming distended. When seen he had a temperature of 102.5°, pulse 110, was emaciated, dyspneic and weak, and had a slight nonproductive cough.

His abdomen was moderately distended with fluid, which shifted. No masses or local tendernesses were to be made out. There was marked dulness in the left chest, up to the third rib in front, and most marked from the third to fifth ribs anterolaterally. In the latter region there was also distant bronchial breathing.

The abdomen was tapped, and 1.5 litres of clear yellowish fluid were withdrawn. It had the general character of an exudate, and an inoculated guinea pig died of generalized tuberculosis. After the withdrawal of the fluid, and while the trocar was still in place, a manometer was attached, but no readings could be obtained on account of the filling of the tube with fluid. A litre of filtered air was introduced through the trocar. One week later 800 cc. of fluid were withdrawn and 500 cc. of air introduced. An attempt was made to aspirate again at the end of another week, but no fluid could be obtained, though it was evident that there was still some there. About this time it was noticed that the dulness in the left chest was increasing, and an attempt was made to aspirate fluid from it, but this was unsuccessful, presumably on account of

[1] Read at the Transatlantic Meeting of the Interstate Postgraduate Clinic Tour, May 26, 1925.

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adhesions, for two days later 650 cc. of fluid was withdrawn from another part of the chest, and 500 cc. of air were introduced. An X-ray taken after this showed a dense shadow extending out from the root of the left lung nearly to the periphery and adhesions connecting this part of the lung with the chest-wall. This density was so great as to make us suspect a malignant growth, but the guinea-pig report dispelled this suspicion. Fluid was withdrawn from the chest and air substituted three times after this, at five to eight-day intervals, each time in diminishing amount.

Both the chest and the abdominal symptoms gradually settled down, so that at the end of four months the patient was practically symptom-free and has now been doing light work for six months.

So far as can be learned, this is the first case on record, on which both a pneumoperitoneum and a pneumothorax have been done.

Case 4: H. L., a boy of nine years, was seen in December, 1923, complaining of vague abdominal symptoms and a temperature of 102°. He had always been delicate, and had long been exposed to a tuberculous mother, who died of her disease shortly after the boy was first seen.

Examination revealed an emaciated, feverish boy with a distended abdomen, from which 1100 cc. of pale yellowish fluid was withdrawn, which showed the characteristics of an exudate, but no tubercle bacilli were found and unfortunately the fluid was not injected into a guinea pig. Eight hundred cubic centimetres of filtered air were injected.

The boy improved rapidly, but the family moved away about ten days later and we were not able to follow the case other than to get a report that he was well six months later.

In addition to the four cases of tuberculous peritonitis reported, I have used pneumoperitoneum in connection with laparotomy in two cases of advanced tuberculous enterocolitis, with apparently some relief but no permanent benefit, as both cases went on to death. In each there was extensive involvement of the lower ileum and colon, to a degree which made resection impossible and enterocolostomy impracticable; therefore, the appendix, which was markedly involved in the tuberculous process, was removed and the abdomen closed. In view of the fact that there was the usual peritoneal involvement over the site of the ulceration, although there was no exudate worth mentioning, it was decided to introduce one litre of air just as the closing of the abdominal wound was completed. This was done with the pneumothorax apparatus, the air being introduced through a blunt needle with a side opening.

One case went on to death in four days, but was apparently much more comfortable than is usual in such cases.

The other lived several weeks, and had three refillings of air at five to seven-day intervals. He pleaded that the air be introduced oftener as he was more comfortable after its use.

Both were advanced pulmonary as well as intestinal cases, and it is not believed that anything was accomplished further than adding to their comfort. Even this is difficult to judge, as it is well known that such cases are often very much more comfortable after operation, and without the use of air. Nor is there the same rationale in its use as there is in peritonitis for, while we know that the air is absorbed from the peritoneal cavity, we have nothing to indicate that air so absorbed would reach the intestinal mucosa any more readily than it would when absorbed by the alveoli of the lungs. However, as there is usually some localized involvement of the peritoneum along with such a process, I believe it is worth a further trial. It should perhaps be used apart from operation since this would furnish a fairer test. This can now be done readily since it has been learned that the parietal peritoneum can be punctured practically with impunity.

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AN ADAPTATION OF THE KOLMER WASSERMANN TECHNIQUE TO TUBERCULOSIS COMPLEMENT FIXATION

J. STANLEY WOOLLEY AND FRANK G. PETRIK[1]

Soon after its invention the remarkable success of the Wassermann test resulted in attempts to apply the principles of Bordet and Gengou to the diagnosis of tuberculosis. Widal and LeSourd, and Camus and Pagniez (1) are reported to have been the first to detect the presence of complement-deviating substances in the blood of tuberculosis patients. Two years later, in 1903, Bordet and Gengou (2) demonstrated the usefulness of the complement-fixation reaction for the detection of antibodies in the serum of tuberculous animals. Since these early attempts many investigators have studied the reaction from the standpoint of its possible value as diagnostic and prognostic aids in clinical tuberculosis. Their findings have been admirably summarized by VonWedel (3), Petroff (4), Wadsworth, *et al.* (5), and by Urbain (6), whose monograph contains an especially complete bibliography. It is noteworthy that in the above summaries, irrespective of antigen or technique, nearly all the investigators obtained a high percentage of positive results in active cases of tuberculosis. Although in principle the laboratory technique employed has been the same as in the Wassermann test, it must be remembered that the reaction is biologically a specific one, as it depends on the presence of a reacting element of the serum, an antibody or reagin, which is specific for tubercle bacilli. In the Wassermann reaction a nonspecific antigen is generally used.

The results obtained have shown at times a wide variation, due mainly to differences in the preparation of antigens and in the technique used. Moreover, the concentration of the circulating antibodies in tuberculosis is never as great as in frank syphilis, and such antibodies are often absent in early and far-advanced cases, the two extremes of the disease. Boez and Duhot (7) state that, if one considers the various stages of pulmonary tuberculosis, the curve of the antibodies, at first low, rises

[1] Loomis Sanatorium, Loomis, New York.

during the first and second periods, that it is maintained and increased at the beginning of the third period, and that at the ultimate phase, in a rapid manner coincident with the onset of terminal symptoms, the antibodies can disappear. For a further understanding of this problem we can do no better than to quote the admirably reasoned hypotheses advanced by Park, Williams and Krumwiede (8):

> The finding of complement fixation in tuberculous patients appears to be dependent on the interplay of two, and possibly three, fundamental factors: (1) The antigens liberated in the body as the result of the invasion of the tubercle bacillus. The exact nature of these is still unknown. They may conceivably consist of the bacillus itself, of products of the disintegration of the bacillus, of endotoxins, or of the products of the disintegration of the tissues evolved during the tuberculous process. (2) The antibodies which may be produced in response to the stimulus exerted by the antigens. (3) We must consider the rôle possibly played by the inhibiting substances that are stated by Calmette and Caulfeild to occur in certain tuberculous serums, and which have the effect of producing negative reactions in serums which contain antibodies. The interaction of these factors in various quantitative relations may give rise to various hypothetical contingencies in the test reactions of a serum, the most important of which appear to be the following:
>
> *Case 1.* No antigen is or has been present in the body, therefore we obtain a negative reaction since there is also no antibody.
>
> *Case 2.* Antigen is present but for some reason there is no antibody formation, and the reaction is negative.
>
> *Case 3.* Antigen is present but the antibody is quantitatively insufficient to more than saturate the antigen; it therefore remains undetected, and the reaction is negative.
>
> *Case 4.* Antigen and antibody are present, the latter in such excess that it can be detected, and a positive reaction results.
>
> *Case 5.* Both are present, but there is also an inhibiting substance which interferes with the reaction of fixation, and therefore the result is negative.
>
> *Case 6.* The antigen formerly present has disappeared and no more is being liberated, but the antibody evoked by it still remains, being demonstrable in decreasing amounts on successive examinations.
>
> Considered in the light of these hypothetical possibilities, the observed discrepancies in the results in examining a series of patients become more reasonable and susceptible of interpretation.

The antigens employed in tuberculosis complement fixation have included simple saline suspensions of tubercle bacilli, alive, autolysed

or heated; glycerine-water bacillary extracts; alcoholic and sodium-hydroxide extracts; tuberculins, and other culture filtrates and antigens consisting of both bacilli and the medium on which the former are grown, depending on the observer. Recently, Wassermann (9) has introduced a "defatted" antigen made from bacilli extracted with a special fat solvent, *tetraline*, and enriched by the addition of a small amount of lecithin. Wassermann claims a strict specificity for his technique.

Opinion has varied as to the true value of the tuberculo-fixation test, depending largely on what success a given observer has had with this or that antigen or technique. Wadsworth, *et al.* (5), who recently have studied the subject very completely, believe that the improved technique in the hands of more recent workers has so greatly reduced the number of positive reactions previously reported in supposedly normal or nontuberculous persons that it is now difficult to exclude the possibility of the reaction being due to some obscure foci of infection. Brown and Petroff (10), in a study of the clinical value of the test, found that patients with a negative test did far better on exercise than those with a positive reaction. They state that, in the determination of what patients need treatment, the test is of more value to the clinician when negative than when it is positive. They also found that the test and the intracutaneous tuberculin reaction do not run parallel courses; the latter is far more persistent. Park, Williams and Krumwiede (8) believe that an apparently normal case giving both a positive complement fixation and a positive Pirquet should be viewed with suspicion. Stoll and Neuman (11) conclude that, with suspicious symptoms and signs, a negative fixation test with their technique increases to a considerable degree the probability of the nontuberculous nature of a given case, the opposite being also true. A positive reaction with neither symptoms nor signs does not justify a diagnosis of active tuberculosis, although it is quite probable that there has been an active process recently. Von Wedel (3) reports that about 70 per cent positive results appears to be the average findings with all types of unselected active tuberculosis cases, for many thousands of fixation tests made by many serologists, using tubercle-bacillus suspensions or tuberculins as antigens. Nevertheless, when the test is used intelligently along with the clinical history, he concludes that the results justify its more extended use. A positive reaction repeated twice seems to prove fairly decisively that the patient has an active tuberculous process. At the other extreme are the findings of Moursund (12). This investigator, using an alcoholic-extract antigen,

obtained only 15.23 per cent positive fixations in 103 cases of active pulmonary tuberculosis, and he concludes that the tuberculo-fixation test, as described in his article, is of no value in diagnosis or prognosis, and is nonspecific.

For the past few years complement-fixation tests for tuberculosis have been done on the blood of nearly all patients on admission to the Loomis Sanatorium, chiefly as a diagnostic aid. Petroff's new antigen (13) was used for these tests, together with a technique similar to the one recently described by him (4). This is a two-tube test, the back tube serving as the serum control. By the use of an excess of amboceptor, as suggested by Aronson and Lewis (14), we later found it possible to dispense with the necessity of allowing for natural antisheep hemolysin, which is a feature of the Petroff technique. Parallel tests convinced us that this simple procedure in no way affected the sensitiveness of the test, and it also seemed to reduce the number of weak (nonspecific?) fixations.

While these tests were being made, the serum would also be tested for the presence of syphilitic antibodies. Several Wassermann techniques have been used by us, the recent one of Kolmer (15) proving by far the most satisfactory, as it rarely gives a false reaction in the presence of a great excess of tuberculosis complement-fixing bodies. Besides requiring ice-box fixation the Kolmer technique utilizes altogether different strengths and amounts of reagents than the Petroff tuberculo-fixation technique. At the same time the possibility of error in the selection of the proper reagent in the setting up of these two tests had to be carefully guarded against. It was plain to us that a technique common to both tests would be of great advantage. In 1924 Kilduffe (16), using the Kolmer Wassermann technique, published the results obtained with tuberculosis complement fixation in a series of 104 cases of tuberculosis. In his work a special antigen was employed which consisted apparently of a boiled water-extract of dried tubercle bacilli, which had been previously subjected to prolonged extraction with lipoid solvents. Complete details of the method of preparation were not published. Results with this technique, in which a Kolmer quantitative test was done on each serum, gave 64 per cent positives, ranging all the way from very strongly to weakly positive. Kilduffe concluded that the quantitative method of complement fixation, as devised by Kolmer for the Wassermann test, is well adapted for use in tuberculosis.

An attempt was made by us to utilize Kolmer's technique as suggested

by Kilduffe's report. For this purpose a boiled water-suspension of ether-extracted tubercle bacilli was used. The results obtained were not nearly as satisfactory as with the Petroff technique (as was shown by parallel tests), either because of the unsuitability of the antigen prepared by us or because tuberculo-fixation does not necessarily give optimum results with ice-box incubation. There is some question about the value of antigens made from defatted bacilli. Wadsworth, *et al.* (5), from a study of the antigenic properties of fractional extracts of tubercle bacilli, believe that the antigenic properties are associated with the acetone-insoluble lipoids, although the bacterial portion, in association with the lipoids, may be a factor, direct or indirect, in the antigenic activities. Wassermann found it necessary to add lecithin, an acetone-insoluble lipoid, to his fat-free antigen. Certainly the presence of lipoids aids in the dispersion of the antigenic material in boiled antigens. Aronson and Lewis (14) found, after complete extraction of tubercle bacilli with alcohol, that the residue was without the slightest antigenic value. With regard to primary incubation, in our hands the warm-bath has always given far better results than the ice-box, irrespective of the antigen used. This is in accordance with VonWedel and others.

In the course of our routine work we had already noted that the original glycerine antigen of Petroff (17), when simplified as suggested by Aronson and Lewis (14), was as satisfactory as Petroff's new antigen and much easier to prepare. With such an antigen and by employing a few changes, the most important of which is the substitution of warm-bath for ice-box fixation, we were finally able to adapt the Kolmer technique to tuberculosis complement fixation, and achieve results in every way equal to those of the Petroff technique. As the technique about to be described has much to commend it, on account of its simplicity and reliability, and particularly where the Kolmer technique is already a matter of routine, we have ventured to present it, with the hope that it may prove useful and helpful, especially to workers in the laboratories of tuberculosis sanatoria to whom its good features may make a special appeal.

Outline of the Loomis-Kolmer Complement-Fixation Technique

This technique is exactly that of Kolmer (Wassermann) except in the following respects:

(1) Two units of complement are used in the test.

(2) Ten units of amboceptor are used instead of two units.

(3) The strength of the antigen (which of course is different) should not be more than one-fourth the anticomplementary unit, and should contain from five to seven (and not more) antigenic units.

(4) The fixation period is two hours in the water-bath at 38°C.

(5) In the test 0.2 cc. of serum is used instead of 0.1 cc. (0.1 cc. of positive serum is used in the antigen titration). Undiluted serum is advised.

(At the end of the article will be found tables taken from Kolmer and changed, where necessary, to suit the altered conditions of the test.)

Technique in Detail

Glassware:

All glassware used in the test should be dry, sterile and chemically clean. Test tubes measuring 0.5 by 4 inches will be found satisfactory, and pipettes having widely spaced graduations are recommended.

Saline Solution:

The distilled water used should not be more than two or three days old. It is brought to a boil and immediately cooled in tap-water. Add 8.5 gm. of sodium chloride, *c.p.*, to one litre of the cooled distilled water, neutralize to pH 7.2–7.5, and "Arnold" for one hour. Ordinary distilled water may have an acidity as high as pH 6, and it is very important to have the salt solution properly adjusted if reliable results are to be obtained.

Complement:

The blood of four or more normal guinea pigs of 500 to 700-gm. weight (fasting for eighteen to twenty-four hours) is collected in sterile, chemically clean, 15-cc. centrifuge tubes. It is allowed to stand at room temperature for one or two hours, then in the ice-box over night, and is finally centrifugated at high speed. Several methods may be used for obtaining the blood. The following is an economical procedure for collecting large quantities: The skin above the external jugular vein is shaved while dry and cocainized, and the hind legs are fastened to a ring on a stand, with the body, head downward, held by an assistant. A small oblique incision, about one centimetre long, is made directly over the jugular; and the vein, exposed by separating the tissues with a blunt scissors, is cut, and the blood allowed to drip directly into a centrifuge tube. A piece of cotton is then pressed against the incision until the bleeding stops, and one stitch is made to close the wound. No special aseptic precautions are necessary. In this manner 7 to 9 cc. of blood may be obtained and the guinea pigs can be bled four times, but not oftener than once every three weeks. After the fourth bleeding they can be used for routine purposes. It is now well known that the serum of an occasional guinea pig is unsuitable for the fixation test for tuberculosis because such complement fails to fix with the serum of tuberculous individuals. There is apparently no

definite relationship between the activity of complement and the fixability of a given specimen of guinea-pig serum; hence the importance of using a pooled complement as described. The complement may be used fresh, or after preservation by the method of Kolmer. It is diluted 1–30 with physiological salt solution for titration.

Hemolysin:

This is prepared by injecting washed sheep red corpuscles into rabbits. Only those sera showing a high titre should be used.

Red Cells:

Fresh sheep blood is definbrinated with glass beads or broken glass rods and then washed six times with saline solution. A two per cent solution is used throughout. Blood preserved with formalin has not yielded satisfactory results in our hands.

Antigen:

The antigen, as previously mentioned, is a simplification of Petroff's glycerine antigen. A four-week-old glycerine-broth culture, of human tubercle bacilli, is washed with tap-water until biuret-free, and thoroughly dried in a desiccator over sulphuric acid. One gram of this is then ground dry in a mortar (under a hood) for one-half hour. A solution of 12.5 per cent glycerine in distilled water is added drop by drop with constant grinding until a smooth emulsion is obtained. This is then diluted with the same solution to 100 cc., and boiled as gently as possible in a small flask with a reflux condenser for one hour. The boiled extract is allowed to stand for three or four hours at room temperature until the larger clumps settle out, and is then pipetted aseptically into 25-cc. pyrex or non-sol flasks, and tightly stoppered. The antigen is then allowed to ripen for at least two weeks at ordinary ice-box temperature before using. It will keep for three months or longer if stored at a low temperature. In view of the high concentration of antigen used in the anticomplementary determinations, the glycerine content was reduced to 12.5 per cent instead of the 25 per cent that the Petroff antigen calls for. A too high concentration of glycerine might make the antigen unduly anticomplementary.

Patients' Serum:

The blood is collected in the usual manner and the serum is inactivated at 55°C. for fifteen minutes. To obtain the best results the serum should not be over twenty-four hours old.

Serum for Antigen Titrations:

(*1*) *Serum for Antigen Unit:* A mixture of four or more, four-plus, fresh, tuberculous sera is used to determine the true antigenic unit of the antigen.

(*2*) *Standard Serum:* A mixed four-plus tuberculous serum is pipetted,

in 0.1 cc. amounts, into small test tubes and stored unheated in a desiccator over sulphuric acid. Before use 0.1 cc. of distilled water is added to each tube and thoroughly shaken until the serum is dissolved. The use of such serum increases the titre of the antigen about 50 per cent. Though this is largely nonspecific, the fixing power of the serum after drying remains constant for months and is used to keep a check on the activity of the antigen.

Titrations

Titration of Amboceptor:

The amboceptor is titrated exactly as in Kolmer's technique for the Wassermann test. The tube that contains the smallest amount of hemolysin giving complete hemolysis with 0.3 cc. of complement is called the unit, and ten units are used in the test. *Example:* If 0.5 cc. of 1–8,000 dilution gives complete hemolysis 1–800 would then contain ten units. (See table 4.)

This large excess of amboceptor is employed in the belief that it results in the dissociation of any nonspecifically bound complement. It also takes care of any natural antisheep hemolysin that may be present in the serum, for with this excess the addition of a unit or so of such hemolysin does not affect the unit of complement. Roger (18) found that, unless the hemolytic antibody is far in excess of that necessary in the Wassermann test, false fixation will occur and the results will be misleading. In regard to this matter Aronson and Lewis (14) state as follows:

> The use of an excess of amboceptor is a fundamental feature of our experimental method. It is based on the conception that the union of antibody and antigen is slow in the case of tuberculosis and that it is in all probability more apt to be involved with various nonspecific factors than in the case of lipoidal reactions with syphilitic material. The amboceptor excess is presumed to dissociate these nonspecific factors and all except the most firmly bound portions of the complement bound to the specific complex, leaving in evidence only the fully completed portion of the specific reaction.

Parallel fixation tests were carried out to determine the value of this feature of the technique, and the results are summarized in table 1.

It is apparent from a study of table 1 that in the normal group the use of an excess of amboceptor, instead of two units, yields results more in accordance with the true state of affairs, suggesting that any fixation obtained with the two units of amboceptor was nonspecific in character. In the tuberculosis group the greater fixation with two units of amboceptor may also be interpreted as due to the influence of nonspecific factors.

Titration of Complement:

The titre of the complement is determined as in table 5. The smallest amount of complement giving complete hemolysis in the presence of the same amount of antigen as used in the test is the unit, and two such units are used.

Example: If 0.3 cc. of complement gives complete hemolysis, it is called the unit of complement, and 0.6 cc. would then contain two units. The complement is then diluted so that 1.0 cc. contains two units.

Titrations of Antigen:

(*a*) *Hemolytic:* The set-up for the hemolytic titration is given in table 6. The tube containing the smallest amount of antigen just beginning to show hemolysis is called the hemolytic unit. The test dose should not be stronger than one-sixth this amount. It is only rarely that the antigen described by us is hemolytic in any strength.

(*b*) *Anticomplementary:* Table 7 contains the anticomplementary titration. The smallest amount of antigen showing some inhibition of hemolysis

TABLE 1

Amboceptor experiment

CLASSIFICATION	NUMBER OF CASES	10 UNITS OF AMBOCEPTOR					2 UNITS OF AMBOCEPTOR*				
		0	+	++	+++	++++	0	+	++	+++	++++
Tuberculous	24	5	1	0	1	17	3	0	1	1	19
Suspected tuberculosis	3	0	0	0	1	2	0	0	0	0	3
Normals	11	10	1	0	0	0	4	1	2	2	2
Totals	38	15	2	0	2	19	7	1	3	3	24

* After the primary incubation 0.5 cc. of red cells was added to each of the control serum tubes and incubated for 30 minutes. The amount of hemolysis in each was noted and the natural antisheep amboceptor was allowed for whenever present.

is the anticomplementary unit. The largest amount of antigen used in the test should *not* be more than one-fourth this amount. *Example:* If the tube containing the 1 to 5 dilution is the unit, then the 1 to 20 dilution is one-fourth the anticomplementary unit. The density of the antigen in the stronger dilutions obscures the reading of the end-point of the reactions. These tubes should always be centrifugated to determine the extent of the inhibition of hemolysis. (See also next paragraph.)

(*c*) *Antigenic:* In table 9 is found the procedure for the antigenic titration of the antigen. The tube containing the smallest amount of antigen giving complete fixation with *0.1 cc.* of a mixture of fresh pooled positive serum and two units of complement is called the unit. From 5 to 7 such antigenic units should be used in the test. If more units are employed some nonspecific fixation may occur, and if less than 5 units are used a smaller percentage of positives may result. *Example:* If 0.5 cc. of 1 to 200 dilution gives complete

fixation, it is called the unit and 0.5 cc. of 1 to 30 would then contain approximately the required amount, and would contain one-sixth the anticomplementary unit. A convenient method for diluting the antigen is given in table 8.

The Test

Table 10 contains details of the test, and the following is a résumé of the necessary steps:

(1) Two tubes containing 0.2 cc. of undiluted serum in each, one receiving 0.5 cc. of antigen (5 to 7 units) and the other serving as the anticomplementary serum control.

(2) Three tubes containing 0.5, 1.0, and 1.5 cc. of antigen, respectively, without serum to keep a check on the anticomplementary power of the antigen.

(3) Wait ten minutes for the union of antibody and antigen to take place.

(4) Add 1 cc. of complement containing two units to all tubes.

(5) Make up the volume in each tube to 2 cc. with physiological salt solution.

(6) Incubate for two hours at 38°C. in the water-bath.

(7) Add 0.5 cc. of amboceptor (10 units) and 0.5 cc. of red cells, and mix thoroughly by shaking each tube individually.

(8) Incubate for forty-five minutes at 38°C. in water-bath.

(9) Read immediately and record results.

Recording of Results:

The results for diagnostic purposes are reported as follows:

0	} =	Negative
+	} =	
++	=	Questionable
+++	=	Moderately positive
++++	=	Positive

Quantitative Test:

To determine the amount of antibodies present in a serum, a slightly modified Kolmer quantitative test can be used. Six tubes containing 0.2, 0.1, 0.05, 0.025, 0.0125 and 0.2 cc. of serum, respectively, are set up. The last tube does not receive any antigen and serves as the anticomplementary control of the serum. The rest of the procedure is the same as in the test and the results are recorded as in the Kolmer test.

Discussion of Technique

The essential features of the technique have been discussed, in part, in the foregoing pages, and the value of an excess of amboceptor has been emphasized. We feel that this feature of the test plays a definite part in the elimination of nonspecific reactions.

Two units of complement are used in the test instead of the amount utilized in the Kolmer Wassermann test. With the shorter period of primary incubation, the deterioration of complement has been found insufficient to require more than this amount. Less than two units were not used on account of the possibility of increasing the number of nonspecific reactions.

The antigen used in the test is easily prepared, and appears to be as sensitive as some of the more complex antigens. It is quite probable that it could be further simplified by omitting the glycerine without any loss of efficiency. The proper concentration of antigen is an important

TABLE 2

Comparison of methods

METHOD	0	+	++	+++	++++	AC*	TOTALS
Loomis-Kolmer.....	43	15	7	17	74	0	156
Petroff.....	42	19	13	13	69	0	156

*AC = anticomplementary.

factor, as complement may not be bound in the presence of either too large or too small a quantity of antigen. Experience has shown this to be an antigen so diluted that the required dose contains from 5 to 7 units. Such a procedure with a satisfactory antigen usually results in a wide anticomplementary range, a most desirable feature in reducing nonspecific fixation. The test dose of the glycerine-extract antigen is usually less than one-fourth the anticomplementary dose. We realize that if each serum could be tested in varying amounts, and with varying amounts of antigen or complement, conditions more favorable for fixation might obtain. But we are describing a two-tube test especially applicable where the Kolmer-Wassermann technique is a part of the routine, and the multiple-tube test is not practicable or desirable.

The amount of serum used in the test, 0.2 cc., was decided upon after failure to obtain optimum fixation with 0.1 cc. of serum, which is what the Kolmer technique calls for. This larger amount was successfully

employed in the tuberculosis complement-fixation tests reported by Besredka, Calmette, Massol and others. An additional reason for doubling the amount of serum was to have a concentration of antibodies equivalent to that in the Petroff technique. As this method has been employed by us for a number of years, it was the standard with which the new technique was compared. Table 2 shows the results obtained in a series of parallel tests, in which the Loomis-Kolmer technique is compared directly with the Petroff method as modified by the use of an excess of amboceptor. From this, the new technique appears to be slightly more sensitive and at the same time shows fewer weak fixations. Also, it will be noted that in the 0.2-cc. amounts the serum was never anticomplementary.

The successful results reported by Kilduffe with ice-box fixation led us to reëxamine this feature of the technique. A number of tests were done in duplicate, one set receiving ice-box and the other warm-bath incubation. The warm-bath invariably yielded superior results. It would be much more convenient if both the Wassermann and the tuberculosis complement-fixation tests could be carried out with the same mode of primary incubation; but it seems advisable at the present time to recommend the water-bath for the latter. The secondary incubation of 45 minutes gives ample time for the dissociation of the nonspecifically bound complement, so that a true picture of the reaction is obtained.

Our method of interpreting results is definitely conservative, chiefly because we have been employing the test for purposes of diagnosis. In this it is very necessary to rule out the influence of nonspecific fixation to arrive at any accurate deduction. Although the use of an excess of amboceptor materially diminishes the amount of weak fixation, a certain portion of which we adjudge to be nonspecific, there is no evidence that it does away with all nonspecific fixation; hence we have seen fit to adhere to the more conservative criteria in reporting results probably influenced in a way by the usual method of interpreting the Wassermann reaction. Normal or nontuberculous persons rarely show marked fixation with the Loomis-Kolmer technique, although somewhat less than 10 per cent of these do give slight inhibition of hemolysis. As it is so difficult to determine in each instance whether such slight fixation is really specific or not, to be on the safe side in the matter of diagnosis we have followed the not unusual procedure of begging the question and assuming that such fixation is nonspecific.

A study of the literature will soon convince one that many observers

have been more generous in interpreting their results, so that comparison of findings, as for instance the percentage of positives, does not always really compare. But few writers state what degree of fixation constitutes a positive reaction, and where a multiple tube test has been used it is not at all easy to collate results. Pinner (19), in a recent article giving results obtained with Wassermann's new antigen, reports any fixation as positive, stating that if all one plus and two plus reactions were to be disregarded the percentage of positive findings in active cases of tuberculosis would not be more than 30 per cent (with this antigen). It is perfectly obvious that the tuberculosis complement-fixation reaction as a diagnostic or supplementary procedure suffers greatly from lack of uniformity and standardization.

Specificity; Negative Control:

Through the coöperation of the Serological Department of the Henry Ford Hospital of Detroit, the Loomis-Kolmer technique was given a trial on the sera of 100 general hospital cases in conjunction with other tests. The results have proved most valuable as a check on the specificity of the technique, particularly as the antigen used was of the same batch employed in most of the findings reported by us. These tests resulted as follows: In the first 100 cases run there were two cases giving three-plus fixation and one a two-plus result. None of these gave any history of tuberculosis, but one of the three-plus cases gave a moderately positive reaction with the Kolmer quantitative Wassermann technique. In addition, there were 9 cases giving a one-plus fixation. Thus, according to our diagnostic criteria, there are only two positive and one questionable reactors in a group receiving general hospital treatment, and, from the very nature of this fact, more apt to have latent tuberculosis than are healthy individuals. This indicates that specific reagin in diagnostic amounts is uncommon among nontuberculous persons, and also shows that the use of such a large amount as 0.2 cc. of serum does not introduce an undue amount of nonspecific elements.

Cross-Fixation

Cross-fixation with this technique does occur with the sera of certain syphilitics. The lipoids of such an antigen as we have described may combine with syphilitic antibodies to fix complement. To circumvent this the use of lipoid-free antigens has been suggested, but from our limited experience such antigens appear to be definitely less sensitive than the whole-bacillus extracts. However, fixation obtained with them would indicate that the antibodies in question were not of syphilitic

origin. Methods of distinguishing between syphilitic and tuberculous antibodies (when present in the same serum) by their different response to prolonged heating have never given satisfactory results. In a further series of tests done at the Henry Ford Hospital, 4 out of 35 cases with a positive Wassermann reaction showed some fixation with the glycerine extract of tubercle bacilli. Only one of these would be considered positive according to our diagnostic criteria. Nevertheless, a positive tuberculosis finding in any Wassermann-reacting serum should always be interpreted with great caution. The serum of tuberculosis patients occasionally gives a false positive Wassermann reaction with a cholesterinized antigen. Such cross-fixation may be avoided however by the use of a plain alcoholic extract antigen in the Wassermann technique. The Kolmer technique rarely gives such cross-fixation.

TABLE 3

General results

CLASSIFICATION	NUMBER OF CASES	TUBERCULOSIS COMPLEMENT FIXATION					POSITIVE SPUTUM	WASSERMANN	PERCENTAGE OF 3 AND 4 PLUS
		0	+	++	+++	++++			
Minimal	13	5	1	0	2	5	5	0	54
Moderately advanced	57	11	4	4	7	31	41	0	67
Far advanced	110	14	7	4	16	69	98	2*	75
Nonclinical tuberculosis	10	6	1	1	0	2	0	0	
Nontuberculous	10	6	2	2	0	0	0	0	
Totals	200	42	15	11	25	107			72

* Sputum positive.

RESULTS OBTAINED IN ACTUAL OR SUSPECTED PULMONARY TUBERCULOSIS

In table 3 are given the results obtained with the Loomis-Kolmer technique on the serum of 200 consecutive cases admitted to the Loomis Sanatorium during the past year. Results were read by one of us who had no knowledge of the patient in question. The classification is based mainly on the roentgenological findings and takes no account of the presence or absence of activity. These blood tests were done soon after admission, and all sera giving negative results, where indicated, were repeated. Hence the negative findings are of very definite value.

The percentage of positive results obtained in the different groups are in accordance with the usual findings indicating the increased produc-

tion of reagin (antibodies) as the disease progresses. Up to a certain point this bears a close relationship with the activity of the disease in the sense that activity is a manifestation of absorption of deleterious products from the focus of infection. Without the production and liberation (absorption) of antigen, reagin would not be formed. Furthermore, there must be an excess of reagin present,—more than enough to saturate the circulating antigen. Some observers have stressed the importance of a positive tuberculo-fixation reaction as an index of the presence of activity. However, there is very little evidence that it possesses any such value. In any individual the presence of a definitely positive reaction would be presumptive evidence of some absorption from a tuberculous focus, but such absorption might be insufficient in amount to give objective or subjective manifestations, namely, clinical activity. It is well known that a positive reaction may persist for months after the disappearance of any signs of clinical activity, but it is difficult to say that this is not due to some response to antigen production, however minute, rather than to the persistence of reagin previously formed. Debré and Lelong (20) have recently shown that tuberculous antibodies transmitted from mother to infant before birth do not persist in the blood of the newborn; they disappear spontaneously in a few weeks. Infants giving such positive fixations did not react to tuberculin and no tuberculous lesions could be demonstrated in those who died. On the other hand, a negative reaction in a tuberculous individual showing no evidences of clinical activity could be interpreted as indicating no absorption. In most instances this is probably true, for patients with negative tests usually stand exercise better than those with positive reactions. But even among them there are marked exceptions, and the possibility of the presence of some inhibiting substance which interferes with the reaction of fixation cannot altogether be disregarded. In the more advanced cases with definite and constant activity the presence of absorption cannot be denied; and the presence of a negative reaction must be explained on other grounds, such as lack of response from exhaustion of the tissues engaged in antibody formation, or saturation of antibodies by the overproduction of antigen.

The presence of a definitely positive reaction in any individual cannot be lightly disregarded. We have indicated that normal or nontuberculous persons rarely give marked fixation; hence the presence of such fixation would point strongly toward the existence of some focus of tuberculosis, perhaps entirely latent as far as clinical signs go. On the other

hand, a negative reaction in a subject patently ill with symptoms and signs suggesting tuberculosis has not, from the very nature and limitations of the reaction, the significance of a positive test. Nevertheless, such a negative reaction increases to a very considerable degree the probability of the nontuberculous nature of the case, particularly if the signs and symptoms are of sufficient intensity to warrant the belief that, if of tuberculous origin, they would indicate definite absorption from some lesion.

There are so many equations entering into the test that a proper interpretation of the findings is not always easy. The better we are acquainted with the pathological processes occurring in a tuberculosis patient and his reaction to his foci of disease, the more valuable will the test appear in the diagnosis or exclusion of the disease. It is a valuable link in the chain of evidence for or against the existence of disease, particularly in the presence of suggestive physical signs not supported by other laboratory evidence. In the past, the lack of uniformity of results from the variety of techniques employed has proved a great obstacle to the general adoption of this helpful reaction. With increasing knowledge and improved methods, many of these difficulties will doubtless be overcome and the reaction become a standard serological method.

CONCLUSIONS

1. The tuberculosis complement-fixation technique (Loomis-Kolmer) described above utilizes, as far as possible, the method of the Kolmer Wassermann test; hence it will be found useful where the latter is a part of the routine.

2. It is as sensitive as the Petroff tuberculosis complement-fixation technique.

3. It rarely gives marked fixation in nontuberculous persons.

4. This feature is believed to be due mainly to the large excess of amboceptor used.

5. Every serum should be given a parallel Wassermann test as cross-fixation with the technique described does occasionally occur with the sera of certain syphilitics. Under these conditions, a positive finding of tuberculosis should be interpreted with caution.

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TABLE 4

Amboceptor titration

NUMBER	COMPLEMENT 1–30	AMBOCEPTOR 0.5 CC.	RED CELLS 2 PER CENT	SALT SOLUTION 0.85 PER CENT	Incubate for 45 minutes in water-bath at 38°C.	RESULTS
	cc.		*cc.*	*cc.*		
1	0.3	1–10,000	0.5	1.7		Slight hemolysis
2	0.3	1– 9,000	0.5	1.7		Moderate hemolysis
3	0.3	1– 8,000	0.5	1.7		Complete hemolysis (Unit)
4	0.3	1– 7,000	0.5	1.7		Complete hemolysis
5	0.3	1– 6,000	0.5	1.7		Complete hemolysis
6	0.3	1– 5,000	0.5	1.7		Complete hemolysis
7	0.3	1– 4,000	0.5	1.7		Complete hemolysis
8	0.3	1– 3,000	0.5	1.7		Complete hemolysis
9	0.3	1– 2,000	0.5	1.7		Complete hemolysis
10	0.3	1– 1,000	0.5	1.7		Complete hemolysis

TABLE 5

Complement titration

TUBE NUMBER	COMPLEMENT 1–30	ANTIGEN 5–7 UNITS	SALT SOLUTION 0.85 PER CENT	Incubate for one hour in water-bath at 38°C.	AMBOCEPTOR 10 UNITS	RED CELLS 2 PER CENT	Incubate for 45 minutes in water-bath at 38°C.	RESULTS
	cc.	*cc.*	*cc.*		*cc.*	*cc.*		
1	0.65	0.5	0.85		0.5	0.5		Complete hemolysis
2	0.60	0.5	0.90		0.5	0.5		Complete hemolysis
3	0.55	0.5	0.95		0.5	0.5		Complete hemolysis
4	0.50	0.5	1.00		0.5	0.5		Complete hemolysis
5	0.45	0.5	1.05		0.5	0.5		Complete hemolysis
6	0.40	0.5	1.10		0.5	0.5		Complete hemolysis
7	0.35	0.5	1.15		0.5	0.5		Complete hemolysis
8	0.30	0.5	1 20		0.5	0.5		Complete hemolysis (Unit)*
9	0.25	0.5	1.25		0.5	0.5		Moderate hemolysis
10	0.20	0.5	1.30		0.5	0.5		Slight hemolysis (Hemolytic complement control)
11	2.00	Omit	0.50		Omit	0.5		No hemolysis

* Two units are used in the test; 0.6 cc. therefore would contain two units. The complement is then diluted by Kolmer's method so that 1 cc. = 2 units.

TABLE 6

Hemolytic titration of antigen

TUBE NUMBER	ANTIGEN 0.5 CC.	RED CELLS 2 PER CENT	SALT SOLUTION 0.85 PER CENT	Incubate for one hour in water-bath at 38°C.	RESULTS
		cc.	*cc.*		
1	Full strength	0.5	2.0		No hemolysis*
2	1–2	0.5	2.0		No hemolysis
3	1–3	0.5	2.0		No hemolysis
4	1–4	0.5	2.0		No hemolysis

* Tubes containing large amounts of antigen should be centrifugated to determine any hemolysis.

TABLE 7

Anticomplementary titration of antigen

TUBE NUMBER	ANTIGEN 0.5 CC.	COMPLEMENT TWO UNITS	SALT SOLUTION 0.85 PER CENT	Incubate for two hours in the water-bath at 38°C.	AMBOCEPTOR 10 UNITS	RED CELLS 2 PER CENT	Incubate for 45 minutes in the water-bath at 38°C.	RESULTS
		cc.	*cc.*		*cc.*	*cc.*		
1	Full strength	1.0	0.5		0.5	0.5		Complete inhibition of hemolysis
2	1–2	1.0	0.5		0.5	0.5		Complete inhibition of hemolysis
3	1–3	1.0	0.5		0.5	0.5		Moderate inhibition of hemolysis
4	1–4	1.0	0.5		0.5	0.5		Moderate inhibition of hemolysis
5	1–5	1.0	0.5		0.5	0.5		Slight inhibition of hemolysis (Unit)*
6	1–6	1.0	0.5		0.5	0.5		No inhibition of hemolysis
7	1–7	1.0	0.5		0.5	0.5		No inhibition of hemolysis
8	1–8	1.0	0.5		0.5	0.5		No inhibition of hemolysis
9	1–9	1.0	0.5		0.5	0.5		No inhibition of hemolysis
10	1–10	1.0	0.5		0.5	0.5		No inhibition of hemolysis

* Tubes containing large amounts of antigen should always be centrifugated to determine any inhibition of hemolysis.

TABLE 8

Directions for diluting the antigen for antigenic titrations

0.2 cc. of 1– 4* dilution plus 1.8 cc. of saline solution	1– 40
0.2 cc. of 1– 6 dilution plus 1.8 cc. of saline solution	1– 60
0.2 cc. of 1– 8 dilution plus 1.8 cc. of saline solution	1– 80
0.2 cc. of 1– 10 dilution plus 1.8 cc. of saline solution	1–100
0.2 cc. of 1– 12 dilution plus 1.8 cc. of saline solution	1–120
1.0 cc. of 1– 80 dilution plus 1.0 cc. of saline solution	1–160
1.0 cc. of 1–100 dilution plus 1.0 cc. of saline solution	1–200
1.0 cc. of 1–120 dilution plus 1.0 cc. of saline solution	1–240
1.0 cc. of 1–160 dilution plus 1.0 cc. of saline solution	1–320
1.0 cc. of 1–200 dilution plus 1.0 cc. of saline solution	1–400

* One of antigen to three of saline solution, etc.

TABLE 9

Antigenic titration of antigen

TUBE NUMBER	4 + SERUM* (4 pooled)	ANTIGEN 0.5 CC.	Wait 10 minutes	COMPLEMENT 2 UNITS	SALT SOLUTION 0.85 PER CENT	Incubate for two hours in water-bath at 38°C.	AMBOCEPTOR 10 UNITS	RED CELLS 2 PER CENT	Incubate far 45 minutes in water-bath at 38°C.	RESULTS
	cc.			*cc.*	*cc.*		*cc.*	*cc.*		
1	0.1	1–400†		1.0	0.4		0.5	0.5		Complete hemolysis
2	0.1	1–320		1.0	0.4		0.5	0.5		Slight inhibition of hemolysis
3	0.1	1–240		1.0	0.4		0.5	0.5		Moderate inhibition of hemolysis
4	0.1	1–200		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis (Unit)
5	0.1	1–160		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
6	0.1	1–120		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
7	0.1	1–100		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
8	0.1	1–80		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
9	0.1	1–60		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
10	0.1	1–40		1.0	0.4		0.5	0.5		Complete inhibition of hemolysis.
11§	0.1	Omit		1.0	0.9		0.5	0.5		Complete hemolysis

* See discussion under standard serum.

† The highest dilution of antigen is given first for convenience in pipetting.

§ Tube 11 is the anticomplementary control of the serum.

TABLE 10

The test

TUBE NUMBER	PATIENT'S SERUM	ANTIGEN (5–7 UNITS)	Wait 10 minutes	COMPLEMENT 2 UNITS	SALT SOLUTION 0.85 PER CENT	Incubate for two hours in water-bath at 38°C.	AMBOCEPTOR 10 UNITS	RED CELLS 2 PER CENT	Incubate for 45 minutes in water-bath at 38°C.	RESULTS
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*		
1	0.2	0.5		1.0	0.3		0.5	0.5		Definite inhibition of hemolysis if positive
2	0.2	Omit		1.0	0.8		0.5	0.5		Complete hemolysis
		cc.								
3	Omit	0.5		1.0	0.5		0.5	0.5		Complete hemolysis
4	Omit	1.0		1.0	Omit		0.5	0.5		Complete hemolysis
5	Omit	1.5		1.0	Omit		0.5	0.5		Complete hemolysis

Tube 2 is the anticomplementary control of the serum.

Tubes 3, 4 and 5 are for the anticomplementary control of the antigen. One set of these tubes should be included in each series of tests performed.

THE STANDARDIZATION OF TUBERCULIN[1]

JOSEPH D. ARONSON

In a former communication (1) the possibility of utilizing the intracutaneous reaction of Mantoux for the purpose of standardizing tuberculin was discussed and it was suggested that the results obtained by this method might be more precise than those obtained by means of the method accepted at present.

The present-day method of standardizing tuberculin is based on the toxicity of tuberculin for tuberculous animals. Donitz (2) has elaborated the method suggested by Koch (3), and considers the minimal lethal dose as that amount of tuberculin which will kill a tuberculous guinea pig in 24 hours. A comparison of the lethal dose of the standard tuberculin with that of the unknown tuberculin is an index of its potency. This method is unsatisfactory, inasmuch as a large number of animals are required, and these vary in their resistance to infection with the tubercle bacillus, in the rate and extent of development of the tuberculous process, and in their sensitivity to tuberculin. Because of these variations the results obtained with any sample of tuberculin may vary and cannot always be duplicated.

To overcome the objections inherent in the method recommended by Donitz, von Lingelsheim (4) suggested that the minimal lethal dose of tuberculin be determined by injecting normal guinea pigs intracerebrally with the tuberculin which had been purified by alcohol precipitation. Neufeld (5) found, however, that this method was not specific, since the same results could be obtained with the alcohol precipitate of sterile glycerine-broth or of sodium sulphate.

The use of the Pirquet method for the determination of the potency of tuberculin has been advocated by Cummins (6). This method has the disadvantage that the amount of tuberculin absorbed cannot be determined. In guinea pigs it is necessary to scarify more deeply than in man, so that the specific reaction is masked by a nonspecific inflammatory reaction due to scarification and to the scratching of the animal.

Since the standardization of tuberculin by *in vivo* methods has proved

[1] From the Henry Phipps Institute of the University of Pennsylvania, Philadelphia.

unsatisfactory because of the variation in resistance to the tubercle bacillus and the marked differences in sensitivity to tuberculin, attempts have been made to develop an *in vitro* method of standardization.

The use of the complement-fixation reaction has been advocated by Watson and Heath (7), who found that the number of antigenic units in various samples of tuberculin parallels the potency as determined by means of the minimal lethal dose.

A strong antiserum was prepared by injecting repeatedly a horse with *B. tuberculosis*. To a constant amount of this serum they added varying amounts of tuberculin, to determine the smallest amount of tuberculin which would be fixed with 1 unit of complement. This amount of tuberculin they define as the unit. They conclude that a tuberculin possessing 750 to 1250 antigenic units per 0.5 cc. of tuberculin is acceptable for the testing of cattle.

Results of the complement-fixation reaction are uncertain because a number of variable reagents are used. For this reason Dreyer and Vollum (8) have attempted to utilize the precipitin reaction to determine the strength of tuberculin. They prepared a specific precipitating serum by repeated intravenous injections of defatted bacilli of Dreyer into a horse. To 5 drops of a 1:4 dilution of this serum they added varying amounts of different dilutions of the tuberculin to be tested. At the same time they made parallel tests with a standard tuberculin which they obtained from the Staats Institut, Frankfurt a/M. To the tubes containing the precipitating serum and the tuberculin normal saline solution was added, to bring the amount in each tube to the same volume. After 9 hours' incubation in a water-bath at 37.5°C. readings were made. The reaction is described as total, standard, trace and nil, the degree of reaction depending upon the clearness of the supernatant fluid, the amount of precipitation and the physical nature of the precipitate. The strength of the tuberculin is calculated from the smallest amount of tuberculin which will give a standard grade of reaction and the smallest amount necessary to produce a trace of reaction. The average of these two determinations is inversely proportional to the average of the determination of the standard tuberculin, which is given an arbitrary value of 100 units. Dreyer and Vollum tested a number of samples of tuberculin by means of the precipitin reaction and controlled their results by the Pirquet skin reaction, applied to tuberculous patients. As a result of this comparative study they conclude that, in general, the two methods give parallel results, although the Pirquet

method may vary considerably in different patients and in the hands of different observers.

To determine the value of the methods proposed for standardizing tuberculin, 9 samples of tuberculin obtained from regular dealers and one sample (no. VII) of standard tuberculin were sent to us from the Bureau of Animal Industry, through the courtesy of Dr. E. C. Schroeder. The minimal lethal dose of these samples was ascertained by the Bureau, and its results were compared with results obtained by means of the intracutaneous method, complement fixation and precipitin reaction.

The intracutaneous standardization was carried out with guinea pigs of 300 to 350-gm. weight, injected intraperitoneally with 0.01 mgm. of a human culture of *B. tuberculosis*. Four weeks later several of these guinea pigs were tested intracutaneously with 0.001 cc. of tuberculin, and were found to give a well-marked reaction. The remaining guinea pigs were divided into separate lots, so that several animals would receive the same samples of tuberculin. The determination of the strength of the samples of tuberculin was made by injecting intracutaneously the same amount of four different samples at widely separated parts of the shaved abdomen. The dosage employed was 0.01, 0.001, and 0.0001 cc. of the tuberculin, diluted with salt solution to a volume of 0.1 cc. The results were read 48 hours later and were designated as A, B, C, or D. The *A* grade of reaction is characterized by necrosis and a marked degree of redness and edema, while the *B* grade shows less marked edema, redness and no necrosis. A moderate degree of edema and inflammatory reaction is designated as *C*, while a slight but distinct degree of edema and inflammatory reaction is termed *D*. In order to further control the intracutaneous method of standardization two tuberculous goats were injected intracutaneously with 0.01 cc. of the different samples of tuberculin. The degree of reaction was determined by comparing the thickness at the site of injection of tuberculin with the thickness at the site of injection of the control concentrated glycerine broth. The difference between these two measurements was considered as the degree of edema. The results obtained by means of the intracutaneous method are shown in table 1.

STANDARDIZATION BY MEANS OF THE PRECIPITIN REACTION

The technique employed to determine the strength of tuberculin by means of the precipitin reaction differed in some of its details from that used by Dreyer and Vollum (8). The antiserum which we used was

prepared by injecting a goat intravenously with from 10 to 50 mgm. of a living culture of an avirulent bovine strain of tubercle bacillus (Bovine iii). The serum was collected carefully, and centrifugated

TABLE 1

SAMPLE NUMBER	TOTAL NUMBER OF GUINEA PIGS INJECTED	RESULTS WITH 0.01 cc. O. T. GRADE OF REACTION				TOTAL NUMBER OF GUINEA PIGS INJECTED	RESULTS WITH 0.001 cc. O. T. GRADE OF REACTION				GOAT XIX 0.01 cc. O. T. DEGREE OF EDEMA	GOAT XX 0.01 cc. O. T. DEGREE OF EDEMA
		A	B	C	D		A	B	C	D		
											mm.	*mm.*
I	4	4	0	0	0	10	8	2	0	0	6	13
II	4	3	1	0	0	10	7	2	1	0	4	13
III	4	0	3	1	0	8	2	2	1	3	2	7
IV	3	2	1	0	0	5	3	2	0	0	2	4
V	4	2	1	1	0	10	2	6	1	1	1	1
VI	3	0	0	3	0	7	0	1	2	4	1	4
VII	5	0	3	2	0	9	0	6	2	1	1	3
VIII	4	1	2	1	0	8	1	3	2	2	1	4
IX	4	0	0	0	4	8	0	0	3	5	1	1
X	3	0	0	0	3	8	0	0	1	7	0	0

Note: With 0.0001 cc. of tuberculin slight reactions were obtained with samples no. I, IV and V in a few animals.

TABLE 2

AMOUNT OF TUBERCULIN	NUMBERS OF SAMPLES									
	I	II	III	IV	V	VI	VII	VIII	IX	X
0.02	+	trace	+	trace	trace	trace	trace	+	trace	+
0.01	++	+	+	+	trace	trace	trace	+	+	+
0.005	++	+	++	++	trace	trace	+	++	++	++
0.002	+	++	+++	+++	+	+++	+	++	+++	++
0.001	trace	++	+	++	+	++	+	+	++	+
0.000,5	trace	+	+	+	+	+	+	+	+	+
0.000,2	trace	trace	+	+	trace	+	+	trace	+	+
0.000,1	trace	trace	trace	trace	trace	+	+	trace	trace	trace
0.000,05	trace	trace	trace	trace	trace	+	trace	trace	trace	trace
0.000,02	trace	trace	trace	trace	trace	trace	trace	0	0	trace
0.000,01	trace	trace	0	trace	0	trace	trace	0	0	0
0.000,005	trace	0	0	0	0	0	0	0	0	0
0.000,002	0	0	0	0	0	0	0	0	0	0
0.000,001	0	0	0	0	0	0	0	0	0	0
0.000,000,5	0	0	0	0	0	0	0	0	0	0

immediately before using, so as to remove the precipitate which often appears in goat serum within 24 to 48 hours after bleeding. The precipitin reaction was carried out by adding 0.2 cc. of the clear immune

serum to amounts of tuberculin varying from 0.02 cc. to 0.000,000,5 cc. After adding sufficient normal saline solution to bring the total volume to 1.0 cc., the tubes were incubated in the water-bath at 37.5°C. for 4 hours, and permitted to remain in the ice-box over night when final readings were made. The reactions were recorded as ++++, +++, ++, +, trace and negative, depending upon the amount of precipitate and the clearness of the supernatant fluid. We have noted that the *trace* grade of reaction shows a faint uniform cloudiness, but no precipitate, and, unless the reaction is carefully compared with the control serum and tuberculin tubes, the reaction may be missed. The precipitin reaction was repeated five times with the results shown in table 2.

The precipitin reactions (table 2) with all of the samples of tuberculin show a well-marked pre-zone, but well-defined reactions are limited to a narrow zone.

TABLE 3

	NUMBER OF SAMPLE									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Antigenic units per cubic centimetre	4000	500	500	1500	2000	1900	5000	150	500	700

STANDARDIZATION BY MEANS OF COMPLEMENT FIXATION

To determine whether there exists any relationship between the lethal dose of tuberculin and the number of antigenic units, as determined by means of the complement-fixation reaction, we employed the technique described in a previous communication on complement fixation in tuberculosis (9). The antiserum employed was the same as that used in the precipitin reaction. The unit value of the serum was first determined by titrating the inactivated serum against a constant amount of a diluted alcoholic extract of tubercle bacilli. To 20 units of this antiserum was then added amounts of tuberculin varying from 0.02 cc. to 0.000,05 cc. After adding 2 units of guinea-pig complement and sufficient salt solution to bring the total volume to 1 cc. the tubes were incubated for 2 hours at 37.5°C., at the expiration of which time 4 units of antisheep amboceptor and 0.1 cc. of 5 per cent sheep-cell suspension were added. Readings were made 30 minutes after the second incubation and final readings were taken the next morning. These titrations were repeated 6 times, and, while the number of antigenic units per cubic centimetre varied, the relative value of each sample of tuberculin remained constant. The results of this study are shown in table 3.

DETERMINATION OF TOXICITY OF TUBERCULIN

The determination of the toxic dose of tuberculin depends on its toxicity for tuberculous animals, and is the method employed by the Bureau of Animal Industry to determine the strength of tuberculin.

The procedure employed by the Bureau, as well as the results of tests made with samples of tuberculin under consideration, were kindly furnished me by Dr. E. C. Schroeder.

To determine the toxicity of tuberculin, guinea pigs are injected with a minute measured quantity of tuberculous tissue obtained from a tuberculous guinea pig. Beginning about three weeks after infection, the sensitiveness of the guinea pigs to tuberculin is tested by injecting intraabdominally one guinea pig daily with 0.375 gm. of old tuberculin per 500 gm. of weight. When this dose causes death within 24 hours, two

TABLE 4

NUMBER OF SAMPLE	DIED IN 24 HOURS	DIED AFTER 24 HOURS	ANIMAL VERY SICK BUT LIVED	ANIMAL SLIGHTY SICK AND LIVED
I	6	0	0	0
II	6	0	0	0
III	6	0	0	0
IV	3	1	2	0
V	4	0	1	1
VI	3	0	3	0
VII	1	2	2	1
VIII	1	0	4	1
IX	0	0	3	3
X	0	0	0	6

guinea pigs are injected daily with 0.25 gm. of tuberculin per 500 gm. weight. The day after it is found that this dose kills one of the two guinea pigs in 24 hours, six guinea pigs are injected with 0.25 gm. tuberculin per 500 gm. weight. If four or more of these die within 24 hours it can be assumed that the remaining animals are sufficiently sensitized, and the remaining guinea pigs are then divided into lots of 6 animals (see table 4). One lot is injected intraperitoneally with 0.25 gm. per 500 gm. weight of the standard tuberculin, while the remaining lots are injected with the same quantity of each sample of tuberculin to be tested.

A tuberculin is regarded as potent if it kills, within 24 hours, at least half of the number killed by the standard tuberculin, and the organs show lesions usually associated with tuberculin intoxication.

The results obtained by the Bureau of Animal Industry are given in table 4. The samples of tuberculin are numbered in the order of their strength.

Samples VI and VII which are of equal strength have standard potency. Samples I and II, which are equally strong, are superpotent; sample III is superpotent but slightly weaker than samples I and II. Samples IV and V, equally potent, are both somewhat superpotent, but less than III. Sample VIII is slightly below standard potency, while samples X and IX are decidedly subpotent, IX having slightly greater potency than X.

TABLE 5

RELATIVE STRENGTH ACCORDING TO TOXICITY	INTRADERMIC REACTION ON GUINEA PIGS		INTRADERMIC REACTION ON GOATS		RELATIVE STRENGTH COMPLEMENT FIXATION	RELATIVE STRENGTH PRECIPITIN REACTION, TRACE GRADE	RELATIVE STRENGTH PRECIPITIN REACTION, + GRADE
	Relative strength with 0.01 cc. O. T.	Relative strength with 0.001 cc. O. T.	Relative strength with 0.01 cc. O. T. Goat No. XIX	Relative strength with 0.01 cc. O. T. Goat No. XX			
I	I	I	I	I	VII	I	VI
II	II	II	II	II	I	II	VII
III	IV	IV	III	III	V	VII	III
IV	V	V	IV	IV	VI	IV	IV
V	VIII	III	V	VI	IV	VI	IX
VI	III	VIII	VI	VIII	X	III	X
VII	VII	VII	VII	VII	II	V	II
VIII	VI	VI	VIII	V	III	X	V
IX	IX	IX	IX	IX	IX	VIII	VIII
X	X	X	X	X	VIII	IX	I

COMPARISON OF RESULTS

To compare the results of the different methods we have arranged in table 5 the samples of tuberculin in their order of strength.

The table shows that a complete agreement between any two of the methods employed does not occur, nevertheless there is a close parallelism between the intracutaneous method and tests made to determine the toxicity of tuberculin.

CONCLUSIONS

This study indicates that the intracutaneous method can be employed to standardize tuberculin, and we suggest the following procedure for this purpose:

Guinea pigs, preferably of a light color, weighing 350 to 400 gm.,

are injected intraperitoneally with a weighed quantity of *B. tuberculosis* or with an emulsion prepared from spleen of a tuberculous guinea pig. Four weeks after infection the sensitivity of the guinea pigs is determined by injecting 0.001 cc. of tuberculin of known strength into the dermis of several of the infected animals. When this dose of tuberculin, after 48 hours, causes a well-marked reaction, as indicated by redness, edema and necrosis, or by redness and edema, the animals may be considered as sufficiently sensitive. The test proper is made by dividing the remaining animals into lots of 3 to 4. The abdomen of each animal is carefully shaved, and all of the animals of each lot are then injected with the same amount of from 4 to 6 different samples of tuberculin. The injections are made intracutaneously in widely spaced areas along the outer edge of the abdomen, in order to prevent the marked edema which occurs when injections are made close to the midline. Each sample of tuberculin should be tested in amounts of 0.01 cc., 0.001 cc., and 0.000,1 cc., made up to a volume of 0.1 cc. with normal saline solution, the injections being made by means of a tuberculin syringe and a 26-gauge needle. Final readings are made forty-eight hours later. A tuberculin which, in a dose of 0.001 cc., will produce distinct edema and redness at the site of injection may be considered as a tuberculin of standard strength.

By the intracutaneous method the strength of a number of samples can be compared under the same conditions, and the result can be followed by the naked eye. On the other hand, determination of the toxic dose is complicated by variation in individual susceptibility of guinea pigs to tuberculosis and of tuberculous animals to tuberculin. Furthermore, intercurrent disease may be a determining factor in producing death. Nevertheless, there is a close parallelism between the intracutaneous reaction and the toxicity of tuberculin. It is probable that the same active principle is involved, differences depending upon the amount used and the method of administration.

SUMMARY

1. The strength of nine samples of commercial tuberculin and one sample of standard tuberculin was determined by means of the intracutaneous injection into tuberculous guinea pigs, and compared with their toxicity for tuberculous guinea pigs, with complement fixation, and with the precipitin reaction. These samples of tuberculin varied greatly in strength.

2. The measurements of relative strength of tuberculins by the intracutaneous method parallels closely the results obtained by determining the toxicity of the same samples.

3. The precipitin reaction and complement fixation have not measured the strength of tuberculins as accurately as the *in vivo* methods.

4. The intracutaneous method is widely used for diagnostic purposes, to determine the sensitivity to tuberculin, and the intracutaneous method of standardization furnishes a direct measure of capacity to produce the skin reaction.

5. Standard tuberculin, in a dose of 0.001 cc. injected into the dermis of tuberculous guinea pigs, should produce distinct redness and edema.

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A CONSIDERATION OF THE EXTENT OF THE NORMAL VARIABILITY OF THE VITAL CAPACITY OF THE LUNGS OF CHILDREN[1]

CHESTER A. STEWART[2]

Because of the present inexact knowledge concerning the extent of normal variability of the vital capacity of the lungs, clinicians experience difficulty in interpreting measurements obtained in individual cases. In order to gain a clearer conception of the normal variability of this measurement during childhood statistical methods have been employed in the analysis of observations upon 2,469 children.

As has been explained elsewhere (Stewart, 1922), if the type of distribution or probability curve for the observations upon the vital capacity of the lungs of children conforms closely to the theoretical symmetrical type of mathematical probability curve, certain deductions concerning normal variability may be drawn having special significance.

To make these deductions it is necessary, therefore, to determine the type of the distribution curve for the observations upon the vital capacity of the lungs. This was accomplished as follows: For each standing-height group (sexes combined, since no sexual difference in variability was apparent) the number of individuals having vital capacities varying 10 per cent, 20 per cent, 30 per cent, etc., above or below the average for corresponding height was determined. From the results obtained in this manner a graph was constructed as follows: On a horizontal scale equal subdivisions or zones were laid off, representing each successive 10 per cent deviation above or below the average vital capacity for corresponding height. On each section of the horizontal scale a rectangle was erected, the height of which equalled the number of individuals having vital capacities falling in each zone. The resulting figure produced in this manner is known as a histogram, which is a graph having certain probability properties. For example, if we now obtain a single additional vital-capacity reading, then, on the basis of

[1] Presented before the Medical Staff of the Lymanhurst School for Tuberculous Children, May, 1925.

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our experience, the probability of the extent of the percentage deviation of this observation from the average for corresponding height is approximately equal to the relation that the area of each rectangle bears to the total area of the histogram. In other words, the probability as to the zone into which this observation will fall is approximately proportional to the area of the zone divided by the total area of the histogram.

A better classification is possible, however, if the number of observations is greatly increased, for it would permit the construction of a histogram of smaller zones. Since, at present, it is not possible to collect additional observations in large numbers, an attempt is made to accomplish the construction of a histogram with smaller zones from the data at hand. This possibility is dependent upon the observation that the different histograms constructed from the data for the various height groups are very similar. At least the data are inadequate to establish any definite features peculiar to any height group. Since this appears to be true, it is considered safe to assume that a histogram representing the distribution of the observations upon the vital capacity for the 2,469 children, combining all the various height groups, is probably fairly representative of the graph for each height group. The construction of the graph from the combined data was accomplished in the following manner:

For each standing-height group the number of individuals (sexes combined) whose vital capacity varied 5 per cent, 10 per cent, 15 per cent, etc., respectively, above or below the average for corresponding height was determined. The number falling within each percentage zone was then totalled, thus combining the individuals of various height groups. These individuals included in each zone total, although varying in height, nevertheless had in common a similar and equal percentage deviation from their expected normal or average vital capacity. Thus, the 394 children having vital capacities ranging between 95 and 100 per cent of the average for corresponding height include individuals of eighteen different height groups ranging from 85 to 90 cm. tall to 175 to 180 cm. tall, inclusive.

From the data combined in this manner a histogram was constructed. On a horizontal scale equal subdivisions or zones were laid off representing each successive 5 per cent deviation above or below the average vital capacity (100 per cent) for corresponding height. On each section of the horizontal scale a rectangle was erected, the height of which

equalled the number of individuals having vital capacities falling in each zone. The resultant histogram is shown in chart 1. This graph,

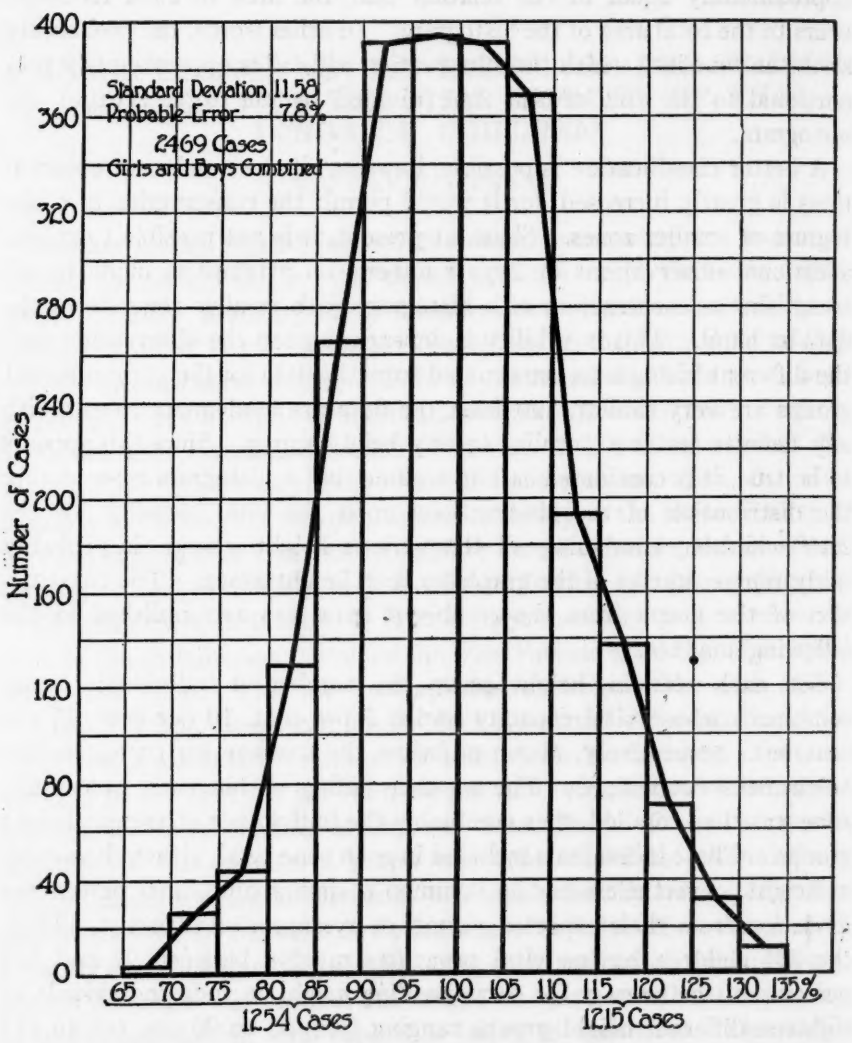

CHART 1. SHOWING DISTRIBUTION CURVE OF THE VITAL CAPACITY OF THE LUNGS OF 2,469 CHILDREN

representing the distribution of the data, has certain probability features as explained before. Should an additional vital capacity measurement be obtained, the probability as to the zone into which this obser-

vation would fall is approximately proportional to the area of the zone divided by the total area of the histogram.

If we now mark the centre of the tops of these rectangles, and draw a smooth curve through these points, we obtain what is termed an approximate frequency or probability curve for the data. Since the area under any section of this probability curve is approximately that under the corresponding part of the histogram, it follows that the curve also possesses the probability property noted for the histogram. The approximate probability curve for the combined data is shown in chart 1. From inspection it appears that the approximate probability or distribution curve for the combined data is sufficiently symmetrical to justify deductions concerning the probability as to the range of normal variability of the vital capacity of the lungs of children. It is known that, when the probability curve for data corresponds to the symmetrical type of mathematical probability curve, under such circumstances the determination of the probable error of the data establishes limits within which about 50 per cent of all observations taken at random can be expected to fall. Also, roughly 25 per cent of the cases will be above and 25 per cent below the limits established by the probable error of the data. Since the probability curve for the combined data, including all height groups, apparently does not differ significantly from those derived for each height group, it is considered safe to assume that the same feature of probability applies to each height group. Consequently, the determination of the probable error of the data for each height group no doubt establishes limits within which approximately 50 per cent of the cases will probably fall were observations obtained upon a large number of comparable cases. The limits of variability established in this manner are probably sufficiently accurate to prove of practical clinical value. If a single observation is obtained, one may thus readily classify the individual as to his standard of physical fitness at least so far as the vital capacity is concerned. Should the individual's vital capacity fall below the limits established by the determination of the probable error for the observations for corresponding height, this individual immediately falls into the group representation of the poorer 25 per cent of the child population. Should the reading fall within the limits of the probable error, the child with respect to the vital capacity belongs to the group representing approximately 50 per cent of the child population, and lastly a reading falling

above the limits established will place the individual in the superior one-fourth of the child population.

The probable error of the data may be computed by means of the formula 0.6745 × S.D., where S.D. is the standard deviation. Since the standard deviation (S.D.) for the observations upon Minneapolis children grouped according to standing height was determined by Stewart (1922), the zone into which 50 per cent of the observations may be expected to fall was easily computed by means of the above formula. This zone is represented graphically in figure 1 of another publication[3] and includes an area ranging in general about 8 per cent above and 8 per cent below the average vital capacity at each standing height. Deviations greater than 8 per cent below the expected normal places a child in a group representative of the inferior one-fourth of the child population in this respect.

At this time it might be well to mention that, while children falling into the inferior 25 per cent group should be carefully examined to exclude disease of the heart or lungs as being responsible for their low vital-capacity reading, nevertheless children representative of the other groups are not necessarily in perfect health merely because the vital capacity is normal, for occasionally this measurement may be normal even in the presence of diseases of the heart or lungs of serious potentialities.

The practical value, then, of the determination of the vital capacity is somewhat limited in connection with the diagnosis of early stages of pulmonary or cardiac disease. Its greatest value probably lies in the fact that changes in the pathological conditions known to exist are reflected by changes in the vital capacity. Thus, by repeated determinations of the vital capacity of the lungs one may follow the course of the disease process by noting its effect upon this measurement. Any progression of the lesion present either in the heart or lungs is immediately reflected by a dimunition in the vital capacity.

In conclusion, it is recommended that physicians employ the determination of the vital capacity of the lungs in the examination of patients having pulmonary or cardiac disease. Such determinations establish the individual's relationship at that time to the expected normal for corresponding height, and will thus permit a classification of the indi-

[3] The vital capacity of the lungs of children having masked juvenile tuberculosis, AMERICAN REVIEW OF TUBERCULOSIS, 1926, xiii, 278.

vidual as to his standard of physical fitness in this respect. Subsequent measurements will prove of special value as an index of the progress of the disease, for any extension of the pathological changes is immediately reflected by a dimunition in the vital capacity below previous determinations. Repeated measurement of the vital capacity, therefore, evidently has a definite prognostic significance. The determination of the vital capacity, however, should not be employed to the exclusion of other methods of examination of the patient, but merely as an additional procedure.

THE VITAL CAPACITY OF THE LUNGS OF CHILDREN HAVING MASKED JUVENILE TUBERCULOSIS[1]

CHESTER A. STEWART[2]

The establishment of the Lymanhurst School for tuberculous children in Minneapolis has afforded an opportunity to make a study of repeated determinations of the vital capacity of the lungs of 248 children having tuberculosis classified as "masked juvenile" in type. The diagnosis for each child was based upon a positive Pirquet tuberculin reaction, undernutrition, an undue tendency to fatigue, occasional slight elevations in temperature of obscure origin, frequent colds and poor general health, exposure to open cases of tuberculosis, and X-ray evidence of enlargement and calcification of tracheobronchial lymph nodes. A few of the children had, in addition, tuberculosis of cervical lymph nodes and of bones and joints. Care was taken not to include any children having active forms of pulmonary tuberculosis, these patients not being admitted to the school.

In table 1 the data (sexes separate) are averaged in groups differing 5 cm. in standing height. Inspection of the table shows that the average initial vital capacity, except for the shortest and tallest individuals of each sex, is constantly below the normal (Stewart, 1922) expected for corresponding standing height. The average percentage of deviation from the normal is apparently greater for the taller groups of children than for the shorter groups. In a few instances the average deviation below normal apparently falls slightly beyond the limits of normal variability, thus placing the individuals in the group representative of the poorer one-quarter of the child population.

After a period of care and treatment at Lymanhurst averaging from 300 to 1000 days during which, as a rule, great improvement in nutrition occurred the vital capacity of the lungs was recorded again (table 1). For both sexes a great improvement occurred, the deviations below the normal vital capacity for corresponding height being greatly reduced.

[1] Read before the Clinical Section at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

[2] Lymanhurst School for Tuberculous Children, Minneapolis, Minnesota.

For practically each group the average final vital capacity was well within the limits of normal variability and was essentially normal, although in general the deviations tended to fall slightly below the normal.

TABLE 1

Showing number of cases, standing height, average initial and final vital capacity, percentage deviation from normal, and period observed at Lymanhurst

NUMBER OF CASES	AVERAGE STANDING HEIGHT	AVERAGE INITIAL VITAL CAPACITY	DEVIATION FROM NORMAL	FINAL STANDING HEIGHT	FINAL AVERAGE VITAL CAPACITY	DEVIATION FROM NORMAL	INTERVAL
			Boys				
	cm.	*cc.*	*per cent*	*cm.*	*cc.*	*per cent*	*days*
5	107	1070	+3.7	115	1240	−1.0	437
6	113	1117	−6.5	120	1400	0.0	426
11	118	1323	−1.1	122	1445	−1.7	288
15	123	1418	−5.5	128	1653	−1.3	369
21	128	1536	−8.2	134	1788	−5.3	333
21	133	1729	−6.7	138	2000	−1.6	301
16	138	1740	−14.4	143	2150	−3.1	356
10	143	1975	−11.0	148	2330	−3.3	378
10	148	2135	−11.4	153	2440	−8.3	315
5	152	2420	−7.3	157	2825	−2.1	259
120			7.6 (average)			−3.0 (average)	
			Girls				
3	103	1000	+12.4	104	1233	+19.7	88
3	108	933	−6.2	118	1167	−8.5	513
8	113	988	−13.0	119	1274	−2.3	362
15	118	1118	−4.6	124	1407	−2.6	432
15	123	1397	−0.12	129	1610	+1.3	425
21	127	1469	−5.8	135	1771	+1.9	448
18	133	1571	−8.0	139	1928	+1.5	348
15	138	1640	−12.1	144	2001	−3.6	324
9	143	1778	−12.7	151	2211	−5.9	407
16	148	2003	−10.2	153	2369	−2.9	311
5	153	2580	+5.8	158	2880	+8.9	309
128			6.7 (average)			0.4 (average)	

The data indicate, therefore, that accompanying the general improvement in the physical condition of the child an improvement in the vital lung capacity also occurred. A graphic representation of the condition existing may be found in chart 1.

TABLE 2

Showing number of cases, average weight, average initial and final vital capacity, percentage deviation from normal, and period observed at Lymanhurst

NUMBER OF CASES	AVERAGE WEIGHT*	INITIAL VITAL CAPACITY	DEVIATION FROM NORMAL	NUMBER OF CASES	FINAL VITAL CAPACITY	DEVIATION FROM NORMAL	INTERVAL
			Boys				
	pounds	*cc.*	*per cent*		*cc.*	*per cent*	*days*
5	40	1120	−0.9				
8	45	1145	−7.5	3	1333	+7.7	296
18	52	1401	−2.2	10	1410	−1.6	235
17	57	1531	−3.2	18	1561	−1.3	356
18	62	1615	−6.9	14	1743	+0.5	312
18	68	1712	−10.4	15	1837	−3.8	355
14	74	1879	−9.3	15	1880	−9.2	335
6	79	1883	−14.5	16	2075	−5.8	304
5	85	2130	−9.8	4	2225	−5.7	343
3	90	2267	9.1	4	2300	−7.7	533
4	95	2575	−1.8	8	2537	−3.3	385
	100			3	2733	−0.76	285
	106			2	2700	−7.4	270
	109			3	2933	−2.4	622
116			−6.7 (average)	115		−3.5 (average)	
			Girls				
7	40	886	−10.5	1	1200	+21.2	285
15	46	1121	−4.7	7	1214	+3.2	411
16	51	1338	+0.8	12	1369	+3.2	314
21	56	1500	+1.9	12	1529	+3.9	452
18	62	1433	−12.1	18	1711	+5.5	383
17	67	1601	−9.2	14	1854	+5.2	331
11	72	1832	−3.8	12	1746	−7.7	301
7	78	1880	−8.1	12	2025	−1.0	475
6	83	2033	−6.3	9	1994	−8.1	470
2	88	2000	−12.7	9	2167	−1.1	364
1	93	2600	+8.1	9	2256	−6.2	405
3	98	2533	+0.9	6	2433	−3.0	308
124	108			1	2800	+5.0	824
	113			2	3150	+15.5	490
			5.0 (average)	24		+0.6 (average)	

* Including clothing.

In order to study the vital capacity of the lungs of these children in relation to body weight, the data were averaged in groups (sexes separated) differing five pounds in weight (table 2 and chart 2). When grouped on this basis, the averages for the initial reading in the majority of instances fall below the expected normal for corresponding body weight. In many instances the deviation below normal apparently is beyond the limits of normal variability, suggesting that the general condition of the child was probably responsible for the subnormal vital lung capacity. After varying periods of considerable length at Lyman-

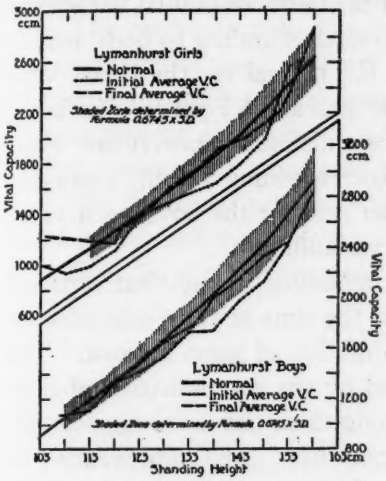

CHART 1

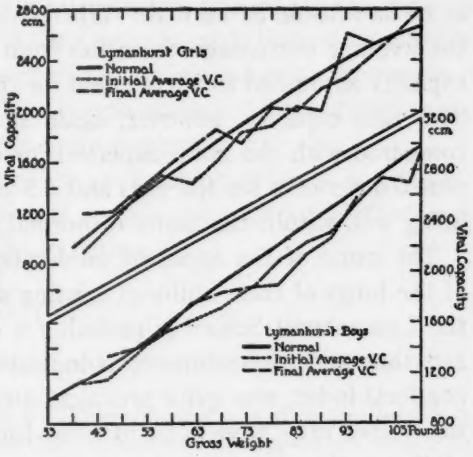

CHART 2

CHART 1. SHOWING INITIAL AND FINAL AVERAGE VITAL CAPACITY OF THE LUNGS OF LYMANHURST CHILDREN BASED UPON HEIGHT AND ALSO ZONE OF NORMAL VARIABILITY AS DETERMINED BY THE FORMULA 0.6745 × S. D.

CHART 2. SHOWING INITIAL AND FINAL VITAL CAPACITY OF THE LUNGS OF LYMANHURST CHILDREN AS BASED ON BODY WEIGHT

hurst the average for the vital capacity, while improved, still averaged somewhat below the expected normal, although in general the deviations below normal were not as great as shown by the averages for the initial vital capacity. Almost without exception the averages for the vital lung capacity following this period of care at Lymanhurst fall within the limits of normal variability, although the general trend is toward the negative side.

In order to determine the extent of the deviations from the normal for the entire group combined (sexes separated) the percentage deviations above and below the average expected were combined, proper

allowance being made for the number of individuals in each group. When combined in this manner the average percentage variance from the normal, for corresponding standing height for the initial vital capacity on entrance to Lymanhurst, amounted to 6.7 per cent for the girls and 7.6 per cent for the boys (table 1). After remaining in Lymanhurst for variable periods of considerable duration the combined average for the vital capacity was only 0.4 per cent below the expected normal for the girls and 3.0 per cent for the boys. For each group at the time of their dismissal from the school the average vital capacity was undoubtedly improved, and so well within the limits of normal variability as to be considered normal. When averaged according to body weight the average percentage deviation from the normal for the initial vital capacity amounted to 5.0 per cent for the girls and 6.7 per cent for boys. The vital capacity, however, again shows distinct improvement when compared with the norm expected for corresponding weight, averaging plus 0.6 per cent for the girls and 3.5 per cent for the boys, each value being well within the limits of normal variability.

The cause of the apparent moderate reduction in the vital capacity of the lungs of these children existing at the time of their admission to the Lymanhurst School is probably a question of some interest. The fact that undernourishment, as indicated by the determination of their ponderal index, was quite prevalent among these children suggests that this factor may have been of some importance. The improvement in vital capacity accompanying the restoration to a more normal level of nutrition probably supports this view. The general prevalence of enlargement of tracheobronchial lymph nodes in this group of patients also may have been a contributing factor, particularly in preventing a complete restoration of the vital capacity to normal during the period of their attendance at Lymanhurst. In this connection may be mentioned that Wilson, *et al.* (1924), found the vital capacity to be reduced in children having enlargement of these lymph nodes. It seems, however that the general improvement in the nutrition and in the physical condition of these children was the more important factor, for at the time of their dismissal from Lymanhurst there was no distinctly noticeable reduction in the prevalence or in the degree of the enlargement of the tracheobronchial lymph nodes.

SUMMARY

1. The vital capacity of the lungs of the 248 children having masked juvenile tuberculosis averaged slightly below the expected normal for corresponding height and weight at the time of their admission to Lymanhurst School for Tuberculous Children. The deviation below normal for the combined group was not beyond the limits of normal variability.

2. Following varying periods of care of considerable duration at Lymanhurst the final average vital capacity of the lungs was distinctly improved, but in general averaged slightly below normal.

3. The moderate reduction in vital capacity present at the time of the admission of these children to the Lymanhurst School was probably due in part to their general undernutrition and probably to the presence of enlargement of tracheobronchial lymph nodes. Of the two factors undernutrition was considered the more important.

4. The beneficial results of the care given these children at Lymanhurst is reflected by the improvement of the vital capacity of the lungs as well as by the improvement in nutrition.

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EDITORIAL

URBAN AND RURAL TRENDS OF TUBERCULOSIS MORTALITY CONTRASTED

When, in 1921, Drolet published his highly instructive composite chart of the tuberculosis mortality of both New York City and the United States for the period 1900–1920,[1] one of its most arresting features was the forceful demonstration that the tuberculosis death-rate of our most populous community was declining much more rapidly than that for the country at large. From an excess of 39 per cent over the rate for the United States in 1900, the rate for New York City had, by 1920, fallen to only 11 per cent above the general rate. Made aware of this occurrence, students of tuberculosis could not help asking, "Is the city yielding its normal and unenviable preëminence as a breeder of tuberculosis? If so, is this the long-looked-for—the first 'near-scientific'—testimony of the effect of social hygiene?"

Since then Drolet has extended this chart from year to year. It has shown us how the tuberculosis rate continued to descend toward that for the nation; so that, within a short two years more, it stood at only 2 per cent above the latter. Here, at a two per cent excess level, it has, from 1922 to 1924, persisted. The Registration Area now embraces so great a proportion of the population (88.4 per cent in 1924) that we may, for practical purposes, regard it as all-inclusive. And, on this basis, we are privileged to view a situation in tuberculosis which has the mortality of a great metropolis at substantially the same figure as that for the immense, almost half-rural fatherland. Within the quarter-century several rather violent changes have become manifest in the habits of tuberculosis. Is it possible that we are on the verge of another *volte-face*, more sensational than any thus far experienced,—of consumption about to flourish best in rural United States?

To the imaginative, Drolet's chart gave only the hint of such a "reversal of form." Perhaps, indeed, the hint was thoroughly misleading. A skeptic would at once point out that the rates for the United States

[1] *Bulletin, New York Tuberculosis Association*, December, 1921. Also reproduced in AMERICAN REVIEW OF TUBERCULOSIS, Abstr. Sec., 1922, vi, 5.

on the chart are those for the expanding Registration Area (from 40.5 per cent of population in 1900 to 88.4 per cent in 1924), while those for New York City are for a relatively fixed terrain and society. As the Registration Area gathered to itself territory of climatic appeal, and therefore much tuberculosis, its tuberculosis death-rate would be bound to undergo a slowing-up of its normal decline. Nevertheless, the chart will show that this explanation of the approach of the two rates will not hold. For instance, in the four years, 1918–1921, the excess of New York City's rate made the extraordinary plunge of from 23 to

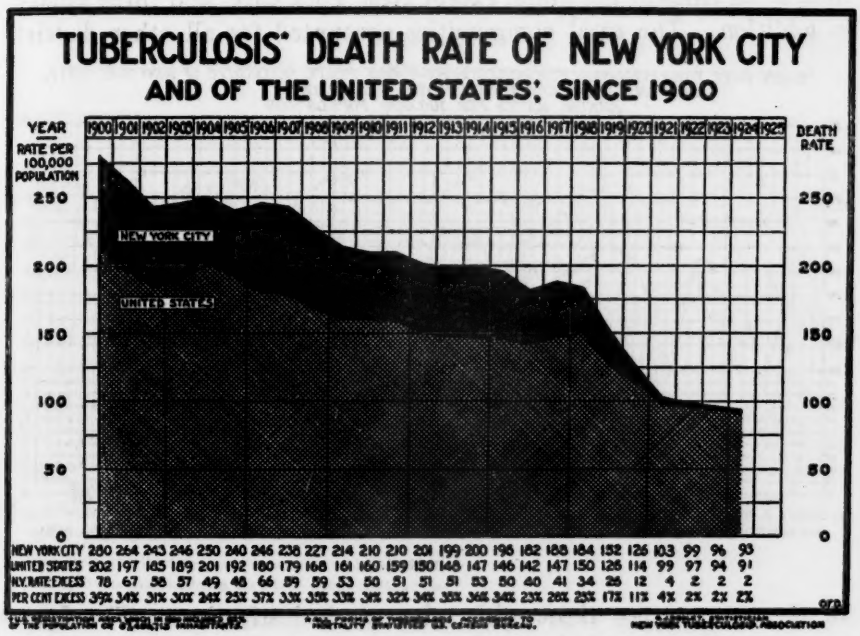

Chart 1. Published through the courtesy of Mr. G. J. Drolet

4 per cent. This, then, was the period of New York City's greatest acceleration of falling death-rate (as compared with the country's). It was also the period of an almost static Registration Area, which in 1918 was 78.5 per cent, in 1919 81.1 per cent, in 1920 82.2 per cent and in 1921 82.2 per cent. One, therefore, is quite likely correct in an opinion that tuberculosis mortality in New York City has been falling much more rapidly than throughout the United States.

Indeed, the phenomenon has been rather general. In 1910 the tuberculosis mortality for fifty American cities of over 100,000 population

was at a rate of 196, or 22 per cent in excess of that for the Registration Area, while in 1920 the rate for the fifty cities was 123, or only 8 per cent above the country's.

Little short of amazing have appeared recent developments in the relation between the urban and rural tuberculosis death-rates in New York State. In 1920 Eichel published for several months graphs which presented the plotting, by months, between 1913 and 1920, of the death-rates from pulmonary tuberculosis for the urban populations of New York State as well as for its rural communities. The urban populations took in all cities of the State, except New York City, and three villages in addition. The rural communities accounted for all other districts

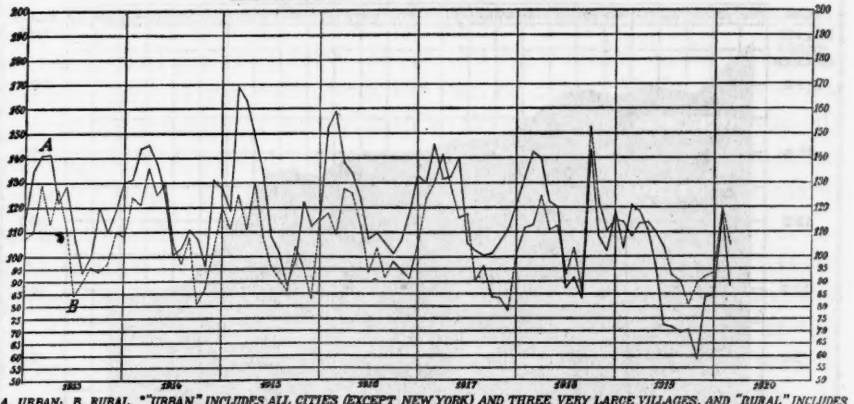
DEATH RATE FROM PULMONARY TUBERCULOSIS: NEW YORK STATE (EXCLUSIVE OF NEW YORK CITY), URBAN AND RURAL AREAS* BY MONTHS, 1913 TO 1920
ANNUAL RATES PER 100,000 POPULATION
A
B
1915
1916
1917
1918
1919
1920
A, URBAN; B, RURAL. *"URBAN" INCLUDES ALL CITIES (EXCEPT NEW YORK) AND THREE VERY LARGE VILLAGES, AND "RURAL" INCLUDES ALL REMAINING DISTRICTS EXCEPT INSTITUTIONAL; AVERAGE ANNUAL RATES, 1913-1917, a, 123.9, URBAN; b, 110.8, RURAL

Chart 2

of the state except those with tuberculosis institutions. The figures were also corrected for deaths in state institutions (Ray Brook). These graphs may be found in issues of the *Vital Statistics Review* of the New York State Department of Health during 1920, and one of them is herewith reproduced as chart 2.

It will be seen that between 1913 and 1917 the average annual urban rate was 123.9 and that for rural districts 110.8. In general, tuberculosis mortality ran "true to form" up until 1917; that is, its rural rate kept consistently (with two or three trifling monthly exceptions) below the urban figures,—a condition that had no doubt obtained ever since tuberculosis had entered the country concerned.

In 1917 rural began to challenge urban tuberculosis to effect, and to

cross the urban line a little more decisively than on former rare occasions. In the early summer of 1918 it moved up to a position even with the urban rate, from which it did not, as formerly, recede. In midsummer it crossed the urban line, to stay ahead of city mortality for practically all of the next eighteen months, or until February, 1920, when the plotting of the figures was terminated. For the greater part of 1919 the rural rate was well above that for the cities, and 1920 opened with the two almost even.

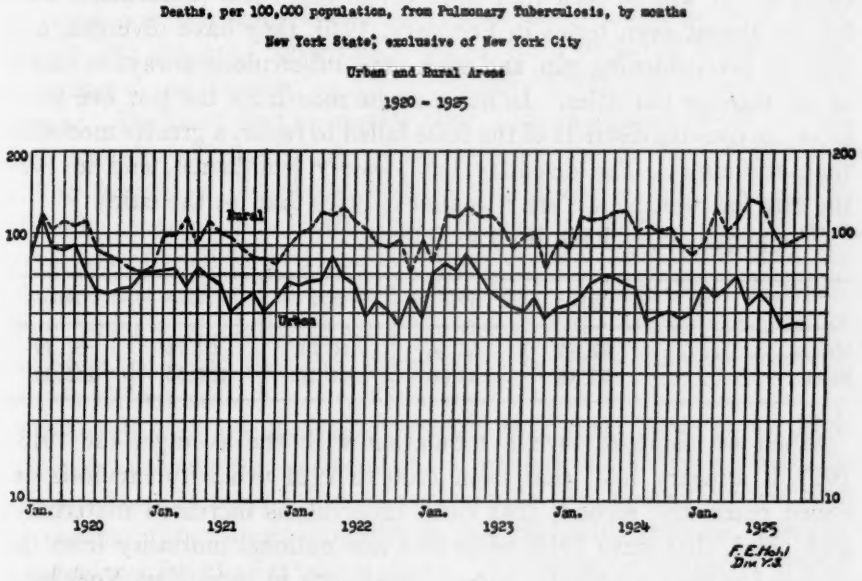

Chart 3. Published through the courtesy of Dr. J. V. DePorte

When these graphs appeared the writer was greatly impressed by their probable meanings. Did they present competent evidence that the country, the rural districts, were slowly overtaking the cities in tuberculosis mortality? Was tuberculosis about to repeat the story of typhoid fever? Or did the graphs merely reflect the resorting of city consumptives, perhaps an increasing tendency, for treatment to the country, there to die and have their cases added to the rural death returns, as coincidently they dropped out of the vital statistics of the cities? At any rate, what was plain was that by 1920 we had reached a condition of greater rural than urban tuberculosis in a state as typical as any of American industrial life.

Through the kindness of Dr. Matthias Nicoll, Jr., Commissioner of the Department of Health of New York State, and Dr. Joseph V. De Porte, Director of the Division of Vital Statistics of the Department, the writer has recently come into possession of data which carry the story of the trends of urban and rural tuberculosis in New York down to date. These show a course which is truly remarkable.

Dr. De Porte has had Eichel's graph (chart 2) continued from February, 1920, to November, 1925, and the extension is shown here as chart 3. It will be seen that since urban and rural tuberculosis were left on almost even terms in February, 1920, they have diverged, and with an ever-widening gap, and with rural tuberculosis always in excess of the rate for the cities. In not a single month for the last five years have the country districts of the state failed to report a greater mortality than the cities. The disparity has steadily lengthened, and in 1924 the rate for rural New York was almost twice that for the cities.

The annual rates have been as follows:

	1920	1921	1922	1923	1924
Urban.....	77.36	63.14	60.08	62.74	57.39
Rural.....	92.84	94.00	101.76	102.50	105.35

When we compare such a tabulation with the averages for 1913–1917, it appears, first, that from 1920 to 1924 urban tuberculosis declined markedly; second, that rural tuberculosis increased materially; and, third, that since 1913, while city and national mortality from the disease has been practically halved, conditions in rural New York have hardly improved.

Of course, what we want to know is whether these death returns actually or substantially reflect what is going on in tuberculosis in the country districts, not only in New York State, but in other states of the nation as well. If, under present conditions of environment and organized propaganda, the trend of tuberculosis is away from our cities and toward our rural sections, it is of enormous importance to discover the fact; as well as whether the country districts of agricultural states and sections are going the same way as those of highly developed industry. Accurate information on these points should give us more exact testimony of the influence of social hygiene than we have ever had heretofore, and will make clearer the directions of future effort. If rural tuberculosis is really holding its own, or increasing, while that in the

cities is retreating, the fact is *prima facie* evidence that the far-flung organizations of the cities are working to effect while rural *laissez faire* is paying the penalty of its inaction.

We may be certain that some of the rural mortality in New York State represents city-dwellers who have gone into the country for treatment and that the urban mortality falls short by a more or less comparable proportion.[2] We have no figures to show, year by year, the actual numbers of country and city residents dead of tuberculosis. Several years ago Eichel attempted a correction of death returns, in order to bring out this information concerning tuberculosis in New York State.[3] He allocated tuberculosis deaths, for a period of twelve years, according to the decedent's place of residence at the time of onset of the disease. He found that in upstate New York an average of 441 persons died annually of tuberculosis whose residence was in New York City, and an average of 184 whose residence was outside of New York State. When he removed these deaths from the upstate figures, he arrived at a correction of from 10 to 15 points lower for New York *State*[4] than the recorded rate and of 3 to 5 points higher for New York City. Furthermore, when he similarly adjusted the rates for the ten largest cities of the State, he found that the "corrected" was higher than the recorded rate in all but two.

Although such results might indicate that rural New York's high rates are misleading as an index of the amount of tuberculosis actually its own, there is other information available to support the view that its resident tuberculosis mortality is declining with extreme slowness, if indeed any fall at all is taking place. The significant feature of chart 3 is the remarkable continuing divergence of the rural and urban lines, as rural mortality rises while that for the cities falls. Were this the portrayal of merely an addition to rural columns of decedents trans-

[2] For instance, the very high tuberculosis death-rates for Sullivan County (Liberty) and Franklin County (Saranac Lake), 630.58 and 544.58 respectively for 1924, are certainly, for the most part, due to nonresident deaths. Again, estimates of more recent years to date have been based on the State Census of 1915 and the Federal Census of 1920, although the State Enumeration of 1925, not yet officially accepted by the Legislature, indicates that some rural counties have grown more rapidly than the urban section. (This information has been furnished by Dr. De Porte.) However, it is hardly likely that these factors are weighty enough to account for the great and increasing divergence between rural and urban rates.

[3] Resident Tuberculosis Deaths, *Monthly Vital Statistics Review, New York State Department of Health*, November, 1922, iii, no. 9, 98.

[4] The reader will note that the correction applies to the figures for New York State (exclusive of New York City) without distinction between urban and rural districts.

ferred and subtracted from their urban residences, its uniformly expanding character could be explained only by the condition of an ever-increasing number of deaths of city-dwellers in the country. Such a circumstance would, at first thought, appear probable because of the undoubted fact that everywhere, and especially in New York State, the period since 1910 or 1915 has been one of the great development and enlargement of sanatorium facilities for the tuberculous, and should therefore have witnessed a continually growing migration of consumptives from city to country, where most sanatoria are placed.

Even though this had been the case it would appear to have had little effect on the distribution of deaths throughout the state. As Eichel showed in 1922, the number of deaths of nonresidents from tuberculosis in upstate New York varied little in the different years from 1916 to 1921, as became apparent in the following tabulation:

Deaths from tuberculosis in New York State (exclusive of New York City)

YEAR	REPORTED	CORRECTED
1916	6,178	5,649
1917	6,490	5,896
1918	6,875	6,141
1919	5,980	5,242
1920	5,408	4,778
1921	4,853	4,328

Eichel also found that the annual number of nonresident deaths formed an almost constant percentage of the recorded deaths. Therefore, what was all-important for our inquiry, *the general trend of the mortality was not affected.*

This trend, it is plain, is a mounting ratio of rural to urban tuberculosis, which has been going on for at least thirteen years, to reach a point at present which puts rural well ahead of urban mortality. During the last five years the trend has been greatly accentuated, as it expresses, no doubt, a static or slightly rising rural rate set against an accelerated urban decline.

As to the causes of this important phenomenon, our present information allows us little more than guesses. Sound interpretation requires much more data that we possess. We should like to have tuberculosis mortality for New York State, urban and rural, and for New York City, tabulated and charted according to age-periods for the last fifteen years

as well as according to permanent residence of decedents. We would also learn much from a similar collation of statistics for other comparable states, such as Connecticut.

Meanwhile, we are justified in the assumption that what data we now have illustrate graphically that the antituberculosis "campaign," immeasurably better carried on in our cities, is beginning to tell, is therefore fundamentally sound and effective, and must be organized to more purpose in the country. Movements such as the Milbank Demonstration in Cattaraugus County and Syracuse will, no doubt, be able to settle definitely several questions that we have raised.

A. K. K.

THE EFFECTS OF ATMOSPHERES RICH IN OXYGEN ON NORMAL RABBITS AND ON RABBITS WITH PULMONARY TUBERCULOSIS[1]

ALVAN L. BARACH

This study was undertaken with the object of determining, in the first place, whether rich oxygen mixtures could be respired over long periods of time without harm to normal animals and, in the second place, whether such mixtures possessed therapeutic value in experimental pulmonary tuberculosis.

Lavoisier (1), soon after his discovery of oxygen in the atmosphere, demonstrated that pure oxygen produced congestion and inflammatory reactions in the lungs. Paul Bert (2) emphasized the harmful effects of high atmospheric pressures on the nervous system. Lorrain Smith (3) pointed out for the first time that the inhalation of air mixtures containing over 80 per cent of oxygen for two to four days resulted in fatal pneumonia. These findings were confirmed by Hill and Macleod (4), who noted, however, a considerable variation in individual resistance to oxygen poisoning. Karsner (5) made a critical study of the pathological histology of the pulmonary changes after exposure to rich oxygen mixtures for one to ten days, and examined also the abdominal viscera and the hematopoietic system. When rabbits were exposed to from 80 to 90 per cent of oxygen for 24 hours no changes other than slight pulmonary congestion were regularly present. At the end of two days widespread pulmonary edema and epethelial desquamation appeared in approximately half the animals studied. After three to four days' exposure these changes were more marked. Fibrin formation and leucocytic invasion were added to the picture, producing varying degrees of pneumonia. The other organs showed no significant alteration other than passive congestion. A few experiments with 70 to 80 per cent of oxygen indicated that minor effects might be produced under these circumstances.

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With the recent use of high concentrations of oxygen clinically, as, for instance, in pneumonia, acute and chronic bronchitis, emphysema, carbon-monoxide poisoning, and cardiac failure, it seemed important to find out whether rich oxygen mixtures could be administered with safety over longer periods of time than were included in the above investigations. In addition, attempts were made to increase the resistance of the pulmonary epithelium by exposing animals to successively higher concentrations of oxygen. Inasmuch as acclimatization occurs in the presence of low oxygen pressures Haldane (6) had suggested that the reverse process might be possible under exposure to high oxygen.

When this portion of the study was completed experiments were undertaken to determine whether experimental pulmonary tuberculosis was influenced by exposure over long periods of time to atmospheres containing high concentrations of oxygen. In 1911 Moore and Williams (7) found that the tubercle bacillus failed to grow in culture media when the concentration of oxygen exceeded 80 per cent, and that after an exposure of one month they were no longer viable. They performed a few experiments on guinea pigs infected with tuberculosis subcutaneously and treated subsequently with oxygen, but noted no differences between the control and the oxygen-treated animals. In 1912 Adams (8) confirmed the lack of growth of tubercle bacilli in high oxygen concentrations, but found that they grew again when exposed to air of normal oxygen concentration. In 1915 Todd (9) reported very favorable results in guinea-pig tuberculosis from the use of ozone and oxygen. On the other hand, David (10) observed unfavorable effects from the use of 40–50 and 80–90 per cent oxygen in pulmonary tuberculosis in guinea pigs and dogs, and beneficial effects from reduced oxygen tensions, such as 10–12 per cent. Recently, Novy and Soule (11) found that the tubercle bacillus would grow in concentrations of oxygen as high as 100 per cent. The optimal concentration they found to be 40–50 per cent; above that limit growth was less abundant.

There are numerous clinical reports on the beneficial action of oxygen when introduced into local tuberculous lesions. Rost (12) described improvement in tuberculous peritonitis, tuberculous joints and psoas abscesses as a result of the injection of oxygen. Similar results were obtained by Stein (13), Bainbridge (14), Smith (15) and others. With the increasing facilities for oxygen treatment, such as oxygen chambers and smaller portable apparatus, the possibility of treating pulmonary tuberculosis in this manner was evident. More information derived from animal experimentation seemed therefore desirable.

METHODS

During the first part of the work a Benedict (16) metabolism apparatus designed for small animals was used. This was soon enlarged by the use of two glass aquaria which were inverted to fit into a water seal. Their size was 14 x 12 x 8 inches. Ventilation was secured by a positive-pressure pump, which delivered air at 12 litres per minute through a bottle of soda lime for removal of carbon dioxide, and calcium chloride for absorption of moisture. Later, an animal chamber was constructed, 5 feet long, 3 feet wide and 3 feet high. It was made of cast iron with a large glass window in the top. The roof possessed a three-inch vertical phlange of cast iron, which sat in a water seal at the upper margin of the chamber. The opening and closing of the chamber took place by raising the lid by means of a pulley and appropriate chains secured to the four corners. The temperature was usually that of the surrounding air. On hot days the air was passed, in addition, through a spiral of copper coils placed in an ice tank. The relative humidity was usually between 50 and 70 per cent. Concentrated sulphuric acid was used as a drying agent because of its greater efficiency than calcium chloride. A Crowell blower was employed which delivered 75 litres of air per minute. The most efficient soda lime for absorption of carbon dioxide was found to be 14–20 mesh, 15 per cent moisture (Wilson).

Oxygen was furnished by a high-pressure tank, equipped with a reducing valve and a gauge especially calibrated to deliver oxygen in litres per minute. In the animal chamber the concentration was raised to 60 per cent by admitting oxygen at 10 litres per minute for 20 minutes, a suitable leak being provided by the opening of a petcock. One to one and a half litres per minute sufficed to maintain the oxygen concentration constant, with a variation usually of less than $\pm$ 3 per cent. The oxygen concentration was analyzed by a simplified method described by Binger (17) which has a maximum error of 0.5 per cent. Higgins and Marriott's (18) dye method of testing carbon-dioxide concentration was used.

The chamber was opened each morning at 9 o'clock and cleaned. Fresh green vegetables, hay or oats, were fed daily. The chamber was usually open for two hours, when the lid was replaced and the oxygen elevated to 60 per cent. In certain experiments shorter periods were used.

Rabbits were employed throughout. In the tuberculosis experiments an effort was made to secure lesions localized as far as possible to the

lungs, producing a progressive involvement ending in death. Intravenous administration of large doses of a human strain of tubercle bacilli in very young rabbits was usually effective. The bovine bacillus in rabbits produced fatal tuberculosis with maximal lesions in the lungs, but also with widespread involvement of other organs. The human strain in small or moderate doses administered to full-grown rabbits was not usually fatal, and did not result in extensive lung lesions. The most successful experiment in fairly young rabbits of moderate size was produced by successive injections of human tubercle bacilli followed by injections of the bovine tubercle bacillus.

In most of these experiments a modification of a method of injection described by Krause (19) was used. The culture grown on Dorset egg-medium was removed by a platinum loop and placed in a glass mortar. The culture tube was weighed before and after removal of growth. The inoculum was then ground with small amounts of water for fifteen minutes, except in experiments 5 and 6, in which it was ground for twenty minutes. Distilled water was added, to dilute the individual dose as a rule to 1 cc. The resulting suspension was used without centrifugation. The experiments were varied from time to time in order to demonstrate whether there was some limit at which the therapeutic measure effectively combated the disease. In the experiment with repeated injections of large doses of a human strain, followed by small doses of a bovine bacillus, the disease ran a chronic course with progressive extension of the pulmonary lesion, ending finally in death. This experiment lasted, in all, seven and a half months, and has more significance from the standpoint of the effect of oxygen on chronic pulmonary tuberculosis than the experiments of one or two months' duration in which death was produced by a massive injection of the human strain or a moderately large dose of the bovine bacillus. In experiments of this character, however, the difficulty of a large and therefore conclusive series of experiments is obvious.

Both the oxygen-treated and the control animals were under close observation, their general appearance and activity being observed daily, their weight being taken as a rule each week and X-ray examinations of the lungs every three to four weeks. When an animal died or was killed the diagnosis of tuberculosis was established by the demonstration of tubercle bacilli in smears of the lung exudate and the typical pathological histology of the section, in some cases by the microscopic picture alone.

The lungs were fixed in Zenker's fluid, embedded in paraffin, and stained with hematoxylin and eosin.

The X-ray films in the early experiments were not satisfactory, partly because difficulties in technique were not yet overcome and partly because the short course of the disease did not permit of a sufficient number of comparisons. Later, the progressive character of the pulmonary lesions was well observed on the films. We wish to thank Dr. Ross Golden for his aid, both in supplying a satisfactory roentgenographic technique and in interpreting the films.

Through the kindness of Dr. Karsner a large number of microscopic sections of the organs of normal rabbits was made available to us, a circumstance which permitted of some reduction in the number of control animals used for comparison. He has called attention in his own work, already referred to, to the frequency of spontaneous lesions of various types in rabbits.

EXPERIMENTS ON NORMAL RABBITS

Experiment 1:

Rabbit, age 5 weeks, male. Kept in small oxygen chamber for one month. Concentration of oxygen maintained between 55 and 60 per cent; carbon-dioxide concentration below 0.6 per cent. Animal removed once a day for three hours, for cleaning cage and feeding. Four control animals followed during this period. There was no change in outward appearance, activity or weight of the oxygen-treated animals. At end of this time animal killed by a blow at the back of the neck. Histological examination made of the lungs, liver, spleen, brain, kidneys, bone-marrow, heart and small intestine. No definitely abnormal changes found in oxygen-treated animal.

Experiment 2:

Two rabbits, both age 4 weeks, one male and one female, weight 500 and 600 gm. Placed in small oxygen chamber for two months. Oxygen concentration maintained generally between 55 and 60 per cent; at times, however, it rose to 70 per cent for short periods. Carbon-dioxide concentration kept below 0.6 per cent. Animals in chamber approximately 21 out of 24 hours. Three rabbits of same age served as controls. There was no difference in weight, general appearance or activity that distinguished oxygen-treated from control animals. Animals killed at end of two months, and routine section of the organs

made as in previous experiment. No significant abnormalities in any organ of oxygen-treated animals as contrasted with control group. In lungs of one of oxygen-treated animals the alveolar walls were thickened in places by mononuclear leucocytes and slight increase in connective tissue.

Experiment 3:

Two rabbits used in this experiment, both males, age 3 months, weight 1200 and 1400 gm. Kept in 60 per cent oxygen in large chamber for 21 to 22 hours of the day for four months. No difference in activity, general appearance or weight from control series of 10 rabbits kept in animal room. These animals were used as part of another investigation and were not autopsied. They were subsequently removed from the chamber and were alive and healthy one year afterwards.

Comment: These three experiments indicate that the inhalation of an air mixture containing 60 per cent oxygen is without any noticeable effect on the general appearance, activity and weight of normal rabbits over periods as long as one to four months. No effect was observed upon their gross and microscopic anatomy for periods as long as one to two months.

Experiment 4:

Rabbit, male, age 3 months,weight 1400 gm. Animal placed in oxygen chamber for seven days, 23 of 24 hours, oxygen concentration ranging between 50 and 80 per cent. Oxygen concentration first adjusted to 50 per cent, and gradually raised to 80 per cent by the maintenance of a small leak. On seventh day animal found dead in chamber. Autopsy negative except for lungs, which showed a diffuse bronchopneumonia, characterized by epithelial desquamation, fibrin, leucocytes, both polynuclear and mononuclear, and a small amount of serous exudate.

Experiment 5:

Rabbit, age 1 month, weight 700 gm. Placed in oxygen chamber 23 out of 24 hours for 12 days. Oxygen concentration maintained between 60 and 70 per cent. Concentration first adjusted to 60 per cent, and gradually raised to 70 per cent during night by maintenance of a small leak. At the end of twelve days death occurred from asphyxia due to accidental turning off of the electricity for two hours. Lungs at

autopsy showed diffuse pulmonary edema. Alveoli filled with homogeneous pink-staining exudate with a few mononuclear cells. In this experiment 70 per cent oxygen appeared responsible for serous exudate into alveoli, which, however, was not in itself fatal.

Experiment 6:

Rabbit, age 4 months, weight 1500 gm. Rabbit breathed 50 to 90 per cent oxygen 23 hours each day. Oxygen was begun at 50 per cent, and through a constant leak gradually elevated to 90 per cent. Animal found dead in chamber at end of seven days. At autopsy lungs congested and heavy. On microscopic section alveoli filled with pink-staining homogeneous exudate. Other organs were negative, except for passive congestion of kidneys and liver.

Experiment 7:

Rabbit, age 6 weeks, weight 600 gm. Animal breathed 60 to 80 per cent oxygen 23 of 24 hours in oxygen chamber. Oxygen begun at 60 per cent, and through a constant leak elevated to 80 per cent. At end of eleventh day animal died. On microscopic section lungs showed diffuse invasion of alveolar spaces by homogeneous pink-staining exudate, and in some places by red blood cells in good state of preservation and a few mononuclear cells. Other organs negative. In this instance the inhalation of 80 per cent oxygen, when gradually built up from 60 per cent, was nevertheless responsible for acute fatal serous pneumonia.

Experiment 8:

Rabbit, age two months, weight 740 gm. Animal placed in oxygen chamber for 23 of 24 hours, with concentration of 60 to 70 per cent. Oxygen begun at 60 per cent, and gradually elevated by constant leak to 70 per cent. At end of three weeks rabbit appeared active and well, and gained weight to 910 gm. Oxygen concentration now elevated to 70 to 80 per cent. At end of three days breathing was noticeably more rapid and labored. Died one week after higher concentration was begun. Lungs in places showed acute serous pneumonia. In other places alveolar spaces obliterated by atelectasis. Adjacent to collapsed alveolar walls were areas of leucocytic infiltration, mononuclear cells predominating. Other portions of lung showed alveoli filled with fibrin, polymorphonuclear leucocytes and epithelial cells. In this case 60 to 70 per cent oxygen was well tolerated for entire three-week period,

but 70 to 80 per cent oxygen resulted in fatal serous pneumonia in one week.

Experiment 9:

Rabbit, age 2 months, weight 755 gm. Animal placed in oxygen chamber 23 out of 24 hours for one month. Oxygen begun at 50 per cent, and gradually elevated by constant leak to 60 per cent at end of 23 hours. At end of one month oxygen changed so as to vary from 40 to 85 per cent. Five days later animal's breathing was rapid and labored. Oxygen concentration was then reduced so as to vary from 40 to 60 per cent, at which it was kept for succeeding 16 days. Labored breathing, however, continued and animal was then killed. During entire period in chamber animal gradually gained in weight from 750 to 1150 gm. At autopsy lungs showed acute serous pneumonia. In this instance exposure for five days at oxygen concentration reaching 85 per cent resulted in fatal pneumonia. A previous exposure to 40 to 60 per cent oxygen for one month did not make animal more resistant to higher oxygen concentration.

Comment: An effort was made to determine whether the lungs might be capable of acquiring some degree of acclimatization or immunity to the effects of very high concentrations of oxygen. The above experiments were performed from two points of view. In the first place the animals were exposed to a safe although elevated concentration of oxygen in the beginning of the day, and then gradually to a higher concentration, that is, 80 to 85 per cent, at the close of the day by the maintenance of a constant leak. In the second place the animals were exposed to a high but safe concentration of oxygen for three to four weeks, following which a higher concentration, namely, 80 to 85 per cent, was instituted. From the use of these two procedures no evidence of acclimatization to higher oxygen was obtained. A concentration of 60 per cent oxygen was, as in previous experiments, well tolerated. In one experiment 60 to 70 per cent oxygen seemed without effect over a period of three weeks. In another experiment 60 to 70 per cent oxygen for 12 days resulted in a serous exudate into the alveoli, which, however, was not fatal to the animal during the experimental period; 80 to 85 per cent oxygen, however, invariably produced a serous pneumonia.

EXPERIMENTS ON RABBITS INFECTED WITH PULMONARY TUBERCULOSIS

Experiment 1:

Ten rabbits, age 8 weeks, weight 700 to 1200 gm., were given each an intravenous injection of 10 mgm. of Saranac Laboratory H37 tubercle-bacillus culture, consisting of a transplant grown three weeks on Dorset egg-medium. Inoculum was prepared after manner described above, 10 mgm. being suspended in 1 cc. of sterile distilled water. Three animals placed in oxygen chamber, one 2 days after injection, the second 9 days after injection and the third 14 days after injection. Animals lived respectively 16, 50 and 25 days after inoculation. Two of control animals died 4 days after injection and were discarded. Of the remainder one died 13 days after injection and another 14 days. Two control animals survived 50 days after injection, and were killed for comparison with oxygen-treated animal that died on 50th day. In this experiment the oxygen concentration varied between 50 and 60 per cent. On four occasions treatment had to be discontinued for periods of 10 to 24 hours because the chamber needed repair. The carbon-dioxide concentration was always below 0.3 per cent. The temperature of the chamber varied between 68° and 78°F., and relative humidity between 50 and 70 per cent. The general appearance and weights of the control and oxygen-treated animals showed no essential differences. Both groups of animals showed miliary tuberculosis, being somewhat more extensive in animals surviving longest and showing no changes incident to inhalation of oxygen.

Comment: In this experiment very young rabbits received a massive intravenous injection of human tubercle bacilli, with the production of extensive pulmonary tuberculosis. Oxygen treatment as carried out had no demonstrable effect on the progress of the disease.

Experiment 2:

Eight rabbits, age 8 weeks, weight 600 to 1000 gm., were inoculated with 10 mgm. of Saranac Laboratory H37 tubercle bacilli. Four rabbits died within 19 days after injection and were discarded. Of remaining 4 animals one was placed in oxygen chamber and 3 served as controls. The three control rabbits died on the following days after injection: 23, 23, 30. The oxygen-treated animal died 39 days after injection. In this experiment the oxygen concentration was 60 per cent for 20 hours each day, occasionally reaching 75 per cent for short periods. In

general appearance and weight the oxygen-treated animal showed no essential difference from controls beyond fact that it lived longer. On section widespread miliary tuberculosis was found in all experimental animals. The lesions of oxygen-treated rabbit were similar to control rabbit dying 30 days after inoculation.

Comment: In this experiment one oxygen-treated rabbit outlived three control animals. The progress of the pulmonary lesion, however, appeared not to be retarded.

Experiment 3:

Fifteen rabbits, age 3 months, weight 800 to 1000 gm., were given an intravenous administration of 8 mgm. of culture of Saranac Laboratory H37 tubercle bacilli. Within the following 11 days 6 animals died and were excluded from the experiment. Of the 9 animals remaining, 3 were treated with oxygen and 6 served as controls. Five of 6 control animals died on the following days after inoculation: 13, 13, 23, 24 and 36. Two of 3 oxygen-treated animals died on following days after inoculation: 22, 77. One control and one oxygen-treated animal were alive 118 days after inoculation and were killed. In this experiment the animals were given 60 to 75 per cent oxygen for 6 hours of the 24. The oxygen concentration was begun at 60 per cent and through the maintenance of a constant leak elevated to 75 per cent at the end of 6 hours. General appearance, weight, X-ray examination and pathological histology of animals revealed no significant changes in the two groups. The control and the oxygen-treated animal that were alive at end of 118 days showed more massive infiltration in lungs than animals dying at earlier period. In each instance widespread involvement of miliary type was present without significant differences between the oxygen-treated and control groups.

Comment: Although 2 out of 3 oxygen-treated animals outlived 5 to 6 control animals there was no beneficial effect to be observed in the general appearance, weight, X-ray examination or pathological histology of the inoculated animals as a result of this type of oxygen treatment.

Experiment 4:

Nineteen rabbits, age 8 weeks, weight 500 to 700 gm., were inoculated with 6 mgm. of a culture of Saranac Laboratory H37. Two animals were treated with oxygen and 17 used as controls. The 17 control animals died on the following days after inoculation: six between

10 and 15 days, five between 16 and 18, and six between 19 and 22 days. One oxygen-treated animal died 20 days after inoculation. The other was crippled by a fall 33 days after inoculation and was killed. In this experiment the animals were given 75 to 80 per cent oxygen for two hours each day. The oxygen-treated animal that had to be sacrificed at 33 days was in somewhat better condition than the others but likewise had extensive miliary tuberculosis.

Comment: One of two oxygen-treated rabbits outlived all the control animals but the tuberculous process was no less advanced. In the second oxygen-treated rabbit no differences were found incident to the inhalation of oxygen.

Experiment 5:

The large animal oxygen chamber was used in this experiment. Twenty rabbits, age 4 months, 900 to 1400 gm., were given an intravenous administration of 8 mgm. of Saranac Laboratory H37 tubercle bacilli. Ten animals were placed in animal chamber and 10 used as controls. The animals were kept in 60 to 80 per cent oxygen 2 hours of the 24. Within the next 33 days 4 of 10 oxygen-treated rabbits and 3 of 10 control rabbits died of pulmonary tuberculosis. No differences in activity, general appearance or weight of the two groups were noted, and the experiment with this type of oxygen-treatment was stopped.

Experiment 6:

The 13 animals remaining from the above inoculation were used in this experiment. Five of the 6 animals that had previously received oxygen were placed in the oxygen chamber. The remaining rabbit formed one of the 8 control animals. These rabbits were older and larger than those employed in former experiments. Intravenous inoculation of a human strain tended to produce a chronic slowly progressive lesion. The first injection consisted, as noted above, of 8 mgm. of Saranac Laboratory H37. The second injection, 5 mgm. of the same culture, was administered 54 days later; the third, 7 mgm. of the same strain plus 0.5 mgm. of Ravenel's bovine strain, 95 days later; the fourth 0.3 mgm. of the same bovine strain, 136 days later; and the fifth, 0.5 mgm. of the same bovine strain, 154 days after the first injection. Naturally, all animals did not survive through the entire inoculation period. (In this experiment the inoculum was ground for twenty minutes.)

The control animals died on the following days after the first injection: 72, 121, 150, 162, 162, 168, 168, 175. Three of the oxygen-treated animals died in the chamber on the following days after the first injection: 157, 168, 188. The last animal was found dead in the chamber in the morning at a time when the carbon-dioxide concentration had reached 5 per cent. All the other animals were markedly dyspneic. It seemed very probable that the death of this animal was due to the carbon-dioxide poisoning, as it had appeared to be in good condition the day before, and its X-ray films had recently shown definitely diminished haziness.

Two of the oxygen-treated rabbits were alive 222 days after the first injection. Both of these had reached higher weights than any of the others. During the last three weeks, however, they began to show signs of dyspnea. When they were removed from the chamber in the morning they became extremely dyspneic, bending their heads directly upward, and breathing in short quick gasps. Their mouths and noses turned a dark bluish color. When replaced in the chamber, and the oxygen elevated to 60 per cent, the head returned to its normal position, the cyanosis disappeared and the dyspnea was markedly lessened although not entirely removed. On the afternoon of the 222nd day the rabbits were removed from the chamber and placed in the animal room. They became progressively more dyspneic and died during the night.

In summary, two of the oxygen-treated rabbits died at about the time the last of the control animals died. The three remaining oxygen-treated animals outlived all the controls, one of them presumably dying of accidental carbon-dioxide poisoning, and the other two dying after removal from the oxygen chamber, over a month and a half after all control animals had died. There was thus distinct evidence of increased length of survival in the oxygen-treated animals, despite a continued series of inoculations with both human and bovine strains of tubercle bacillus.

If the weights of the animals are compared (see table 1 for complete data) it will first be seen that the control animals were on the whole heavier than the oxygen-treated animals. Nevertheless, the two surviving rabbits achieved higher weights than any of the controls. Thus, one went from 1050 gm. to a maximum of 2850 gm., and the other from 960 to 2550 gm., whereas the two highest control animals went from 1500 to 2250 gm. and from 1500 to 2350 gm. The remaining rabbits in the two groups showed no significant differences in weight.

TABLE 1

Experiment 6: Experimental pulmonary tuberculosis. Five rabbits were treated with 60 per cent oxygen and eight were untreated. Data on length of life, weight and pathological histology

DATE	INTRAVENOUS INJECTION OF TUBERCLE BACILLI	WEIGHT OF CONTROL RABBITS (GRAMS)								WEIGHT OF OXYGEN TREATED RABBITS (GRAMS)					Remarks	PATHOLOGICAL DATA
		No. 3	No. 5	No. 11	No. 13	No. 15	No. 17	No. 18	No. 20	No. 2	No. 8	No. 9	No. 12	No. 16		
7/30	8 mgm. human	1040	1150	1400	1560	1590	1500	1500	1470	920	1050	960	1350	1320		*Controls:*
8/8		1020	1080	1290	1510	1500	1520	1530	1360	970	950	880	1240	1310		*No. 3:* Infiltration of tuberculous granulation tissue and confluent tubercles. Caseation in centres
8/18		1090	1110	1400	1650	1570	1660	1590	1550	970	1150	1040	1370	1350		*No. 5:* Tubercles of various size scattered throughout lung, some forming large confluent masses
8/22		1210	1110	1510	1640	1580	1820	1700	1590	940	1190	1040	1430	1440		*No. 11:* Large masses of tubercles infiltrate entire lung, very little alveolar structure remaining
9/3		1270	1220	1580	1660	1620	1860	1840	1610	970	1210	1140	1390	1480		*No. 13:* Numerous small tubercles are present throughout lung; in places they form large caseous masses
9/16		1110	1330	1650	1940	1550	1940	1820	1920	1110	1330	1460	1460	1760		*No. 15:* Tubercles of various size scattered throughout lung, some of them fusing to form large caseous masses
9/23	5 mgm. human	1060	1240	1450	1900	1400	1760	1760	1950	1090	1180	1230	1640	1670		*No. 17:* Tubercles of various size scattered throughout lung. In places the alveoli are distended, the walls broken and thinned out
10/1		1300	1450	1560	1820	1430	1900	1920	1850	1190	1240	1375	1580	1740		*No. 18:* Widespread infiltration of tubercles of various size, some show caseous centres
10/9		1155	1525	1795	1795	1295	1825	1995	1945	965	1196	1345	1450	1570		*No. 20:* Widespread infiltration of tubercles of various size, some showing caseous centres
10/15		1130	1430	1670	1810		1810	2100	1830	1010	1320	1450	1500	1680	No. 15 died 10/11/24	*Oxygen-treated rabbits:*
10/23		1110	1590	1860	1800		2150	2300	2100	1080	1400	1540	1540	1760		*No. 2:* Large confluent tubercles infiltrate entire lung. In places the centres show caseation
10/30	0.5 mgm. bovine	1140	1660	2100	2050		2250	2350	2150	1210	1575	1730	1500	1950		*No. 8:* Large confluent tubercles the centres of which frequently show caseation widely diffused through lung. In places the alveoli contain a homogeneous pink-staining exudate
11/4	7 mgm. human	1090	1640	1900	1850		2000	2300	1900	1400	1900	1950	1375	1725		*No. 9:* Large confluent tubercles with marked caseation throughout. There is considerable new connective tissue which replaces the alveolar structure
11/14		1000	1750	1900	1900		1950	2100	1925	1600	2100	2250	1300	2200		*No. 12:* Large tubercles with caseous centres scattered through lung; in places the alveoli are thickened by invasion of tuberculous granulation tissue. The alveoli are frequently broken and form large air-spaces
12/1			1800	1950	1950		1900	1950	2100	1600	2250	2350	1350	2000	No. 3 died 11/29/24	*No. 16:* Widespread infiltration and confluent tubercles with central caseation. In places tuberculous granulation tissue invades the alveoli
12/15	0.3 mgm. bovine		1800	1950	2050		1975	1925	2000	1700	2275	2250	1350	2000	No. 18 died 12/28/24	
1/2	0.5 mgm. bovine		1800	2125	2000		1875		1850	1725	2360	2200	1300	1900	No. 12 died 1/4/25	
1/14			1625	2050	1850		1650		1850	1650	2700	2200		1650	No. 2 and No. 13 died 1/15/25	
1/25											2850	2400		1550	No. 11 died 1/16/25	
1/29											2700	2550		1600	No. 5 and No. 17 died 1/19/25	
2/6											2650	2500			No. 20 died 1/22/25	
2/16											2700	2500			No. 16 died 2/4/25 (5 per cent CO_2)	
2/25											2600	2300				
3/4											2300	2100			3/10/25: Nos. 8 and 9 taken out of O_2 chamber and died during the night	

The X-ray pictures in this experiment are of considerable interest. Those of the control rabbit, no. 13, and the oxygen-treated rabbit, no. 9, are reproduced, but the detail of the films is unfortunately partially lost in the photograph. A description of the lesions as found on the films is given in the legends accompanying the photographs (figs. 1 to 9). The findings may be summarized as follows:

In both the control and oxygen-treated animals the first signs of infiltration appeared three and a half months after the first inoculation. The films at this date were clear except for one or two small areas of diminished density in the lower lobes, so minute as to cast doubt on their significance, were it not that unmistakable infiltration appeared in these regions in later films. Four and a half months after the first inoculation the films of both the control and the oxygen-treated animals showed scattered areas of diminished density, approximately 2 by 3 mm. to 3

Description of figures 1 to 6

FIG. 1. RABBIT 9

Experimental pulmonary tuberculosis, treated with oxygen. X-ray film three and a half months after first inoculation is clear except for a doubtful area of diminished density 2 mm. in diameter in left lower lobe and an irregular area 3 by 5 mm. in right lower lobe, which seems to consist of three confluent areas approximately 2 mm. in diameter.

FIG. 2. RABBIT 9

Experimental pulmonary tuberculosis, treated with oxygen. X-ray taken four and a half months after first inoculation shows an irregular area of diminished density in each lower lobe approximately 3 by 5 mm.

FIG. 3. RABBIT 9

Experimental pulmonary tuberculosis, treated with oxygen. X-ray taken five months after first inoculation shows scattered areas of diminished density varying in size from 2 by 3 mm. to 3 by 5 mm., more marked in right lower lobe.

FIG. 4. RABBIT 9

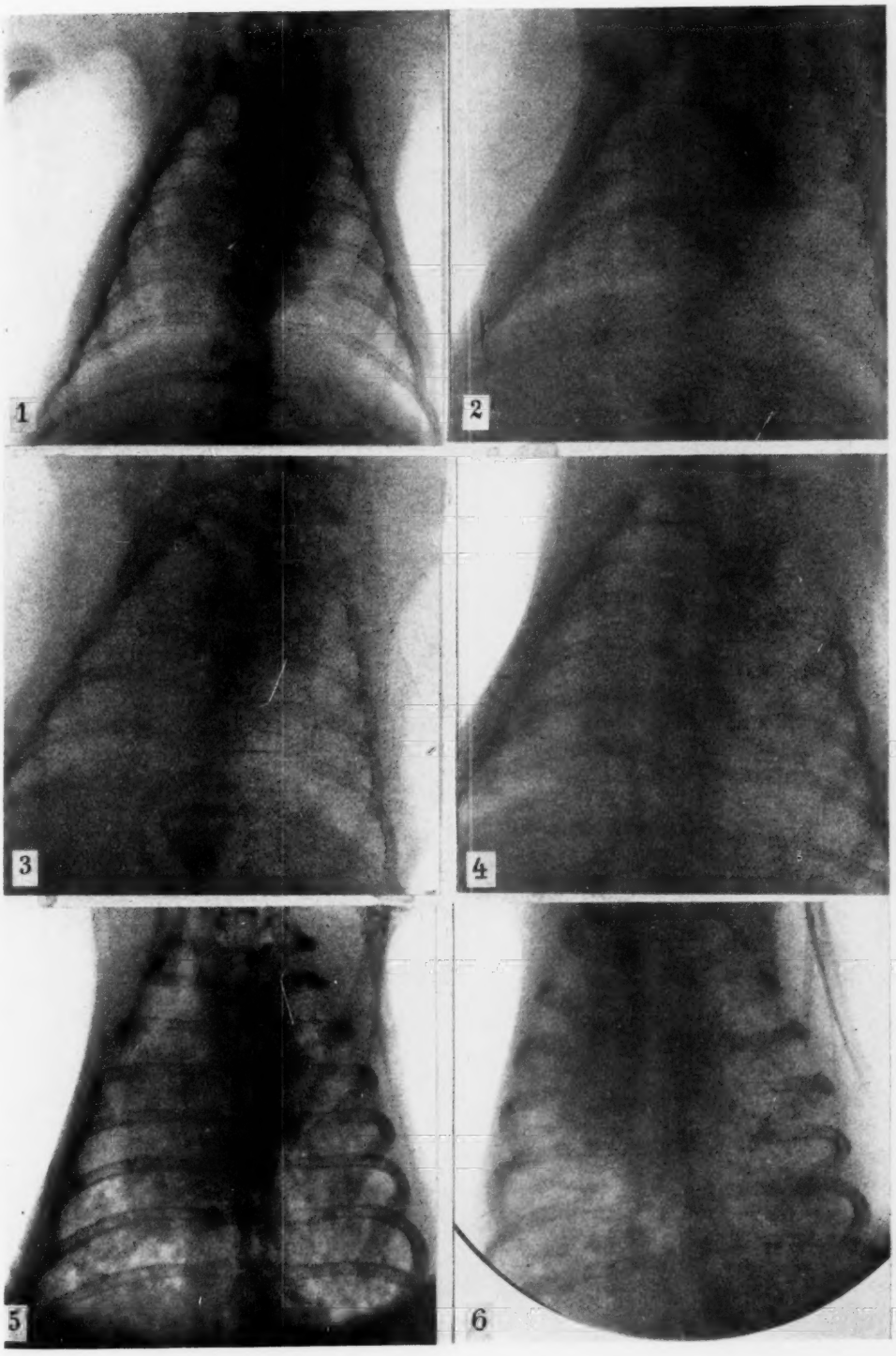
Experimental pulmonary tuberculosis, treated with oxygen. X-ray picture taken six months after first inoculation shows scattered areas of diminished density varying in size from 2 by 3 mm. to 3 by 5 mm., more numerous than in previous film.

FIG. 5. RABBIT 9

Experimental pulmonary tuberculosis, treated with oxygen. X-ray taken six and a half months after first inoculation shows widespread infiltration of both lungs, more marked on right. Areas of diminished density are irregular in shape, size varying from 2 by 3 mm. to 3 by 5 mm.

FIG. 6. RABBIT 9

Experimental pulmonary tuberculosis, treated with oxygen. X-ray film taken seven and half months after first inoculation, on day preceding death, is almost white with infiltration. Confluent areas of diminished density occupy both lungs so that no separation of single lesions are made out.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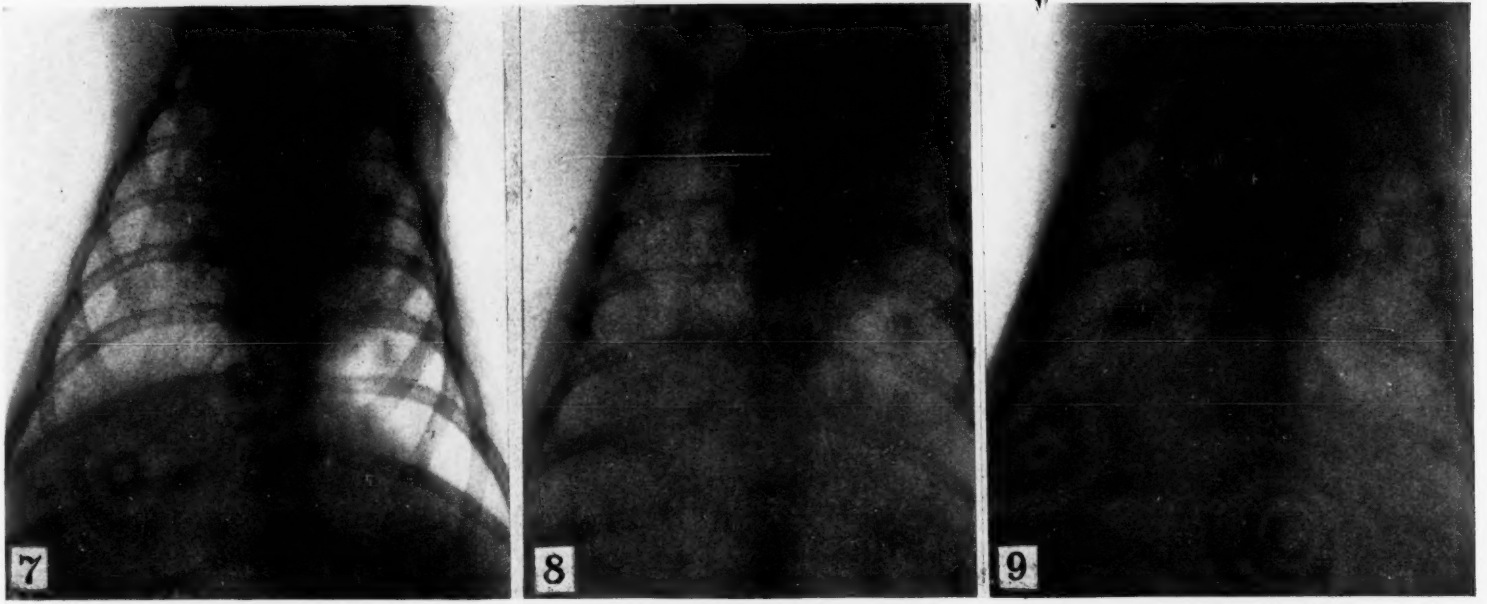

FIG. 7. RABBIT 13

(Experimental pulmonary tuberculosis, untreated.) X-ray taken three and a half months after first inoculation is clear except for a doubtful area of diminished density 1 mm. in diameter in right lower lobe and an area 2 by 3 mm in left lower lobe.

FIG. 8. RABBIT 13

(Experimental pulmonary tuberculosis, untreated.) X-ray taken four and a half months after first inoculation shows scattered areas of diminished density, 2 by 3 mm. to 3 by 4 mm. in size, present in both lungs.

FIG. 9. RABBIT 13

(Experimental pulmonary tuberculosis, untreated.) X-ray film five months after first inoculation is hazy throughout. There are numerous areas of diminished density, varying in size from 2 by 3 mm. to 4 by 7 mm., scattered irregularly through both lungs.

by 5 mm. in size. Due to the large size of the rabbit's heart these lesions are more visible at the base and periphery of the lung than at the apex. Five months after the first inoculation the lesions are more numerous and larger. The size varies from 2 by 3 mm. to 4 by 7 mm. Although the infiltration is scattered throughout both lungs, the lesions are generally discrete and a considerable portion of the lungs in between appears uninvolved. No differences are made out between the control and oxygen-treated animals. Six months after the first inoculation all the 8 control animals and two of the 5 oxygen-treated animals are dead. The remaining 3 oxygen-treated animals show widespread infiltration of both lungs, the lesions being more numerous and more extensive than on previous films. Seven and a half months after the first inoculation only 2 of the oxygen-treated animals are alive. The films are almost white with widespread confluent infiltration of lesions of varying size. Discrete areas of diminished density are no longer visible, but the infiltration is plainly seen to be due to the joining of innumerable small lesions of the size found in previous films.

The X-ray pictures indicate that 3 oxygen-treated rabbits developed more extensive pulmonary tuberculosis than the 8 control rabbits which they outlived. The 2 oxygen-treated rabbits that died earlier showed the same degree of tuberculosis as the control animals that died at that time. The extent of tuberculous infiltration, according to the X-ray data, seemed to be in proportion to the length of life of the animal irrespective of the inhalation of oxygen. In all instances, tubercle bacilli in great numbers were recovered from the stained smear of the lung exudate.

The pathological material offered a general confirmation of the X-ray evidence. The animals dying in the earlier period showed less marked invasion of the lung than those dying later. The character of the pathological changes was invariably that of miliary tuberculosis. Tubercles of varying size were seen on microscopic section. In the animals dying late the tubercles were more confluent and with larger central areas of caseation, tuberculous granulation tissue was more common, and emphysema of the remaining alveoli was noticeable. This latter condition was especially marked in the 2 oxygen-treated rabbits that survived seven and a half months. The alveolar walls in places were thinned out and broken, forming large air-sacs. No differences in the control and the oxygen-treated animals were made out other than those explainable on length of survival.

The appearance of the surface of the lung is shown in the accompany-

ing illustration (fig. 10) from rabbit 13, a control animal. The surface is seen to be studded with tubercles which are raised above the surface of the uninvolved lung, measuring between one and three mm. in size. The microscopic section of the same animal (fig. 11) shows the changes described above. The microscopic picture of no. 9, one of the oxygen-treated animals (fig. 12), shows lesions similar to the control, with larger conglomerate tubercles and more extensive caseation. Further comment on this experiment will be reserved for the discussion on results.

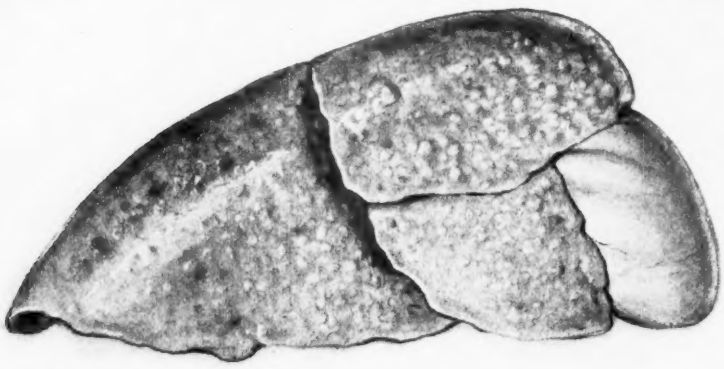

FIG. 10. EXTENSIVE MILIARY TUBERCULOSIS OF THE LUNG IN CONTROL RABBIT 13

Experiment 7:

Thirteen rabbits, weight 850 to 1300 gm., age 3 months, were injected with 0.2 cc. of an emulsion of bovine tubercle bacilli, Ravenel strain, prepared by grinding the inoculum for fifteen minutes and suspending it in sterile distilled water. Eight rabbits were used as control animals, and 7 were put in the oxygen chamber at a concentration of 60 per cent for 22 hours of the 24. The control animals died on the following days after inoculation: 27, 29, 29, 31, 31, 36, 36, 36. The oxygen-treated animals died: 21, 29, 29, 31, 35, 36, 41. No differences in weight of the control as opposed to the oxygen-treated animals were found, all showing a distinct drop after the third week. No differences in appearance or behavior of the two groups were manifest. The X-ray pictures taken on the 29th day after the injection showed a very extensive mottling of both lungs with small confluent lesions in all the surviving animals, irrespective of treatment with oxygen.

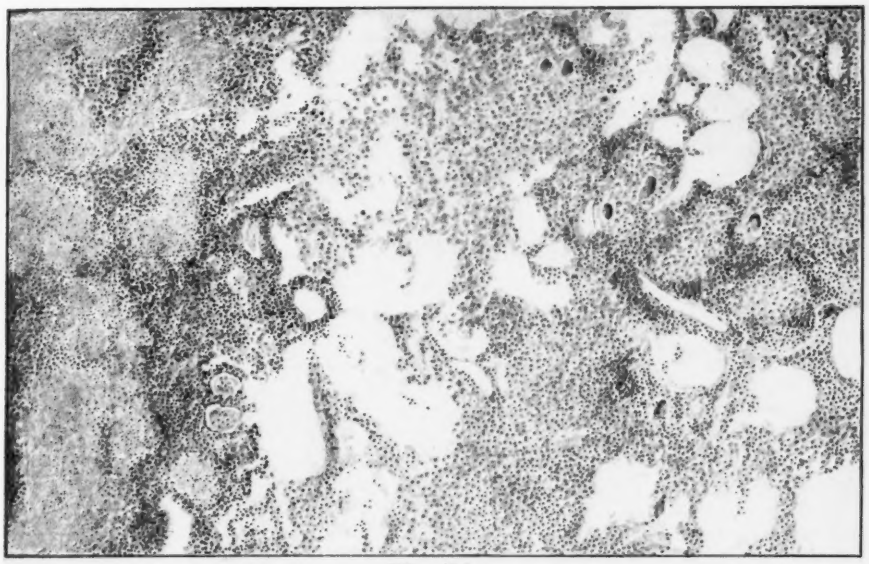

Fig. 11

Fig. 12

FIG. 11. MICROSCOPIC PICTURE OF MILIARY TUBERCULOSIS OF THE LUNG IN CONTROL RABBIT 13

FIG. 12. MICROSCOPIC PICTURE OF MILIARY TUBERCULOSIS OF THE LUNG IN OXYGEN-TREATED RABBIT 9

At autopsy the surface of the lung was everywhere studded with tubercles of 1 to 3 mm. in size. On microscopic section confluent tubercles of varying size were seen throughout both lungs. Large areas of caseation occupied the centres. The stained smear of the lung exudate showed enormous numbers of tubercle bacilli. No differences in control and oxygen-treated animals were present.

Gross tubercles were found scattered through the other organs, particularly the liver, spleen, kidneys and intestine. Microscopic section was not made of any extrapulmonary lesions.

Comment: In this experiment an acute overwhelming miliary tuberculosis was produced by the intravenous injection of a large dose of bovine tubercle bacilli. Only one of the oxygen-treated animals outlived the control group. The extensiveness of the lesion, according to the X-ray picture and the pathological examination, was not different in the two groups. The behavior, appearance and weight changes of the control and oxygen-treated rabbits showed no significant differences.

DISCUSSION OF RESULTS

The experiments in the first part of the paper showed that rabbits kept in a chamber containing 60 per cent oxygen for four months suffered no alteration in appearance, behavior, weight or growth. At the end of one and two months histological section of the body organs revealed no abnormality. The production of acute serous pneumonia by the inhalation of 80 to 90 per cent oxygen for two or more days was confirmed. In addition, it was demonstrated that no increase in resistance of the pulmonary epithelium could be brought about by previous exposure to oxygen concentrations varying between 40 and 70 per cent for periods of one to three weeks. The border-line of safety appeared to be 70 per cent, which in most instances was without harmful effect. In one animal, however, exposure to 70 per cent oxygen for twelve days caused pulmonary edema. The results indicated that 60 per cent oxygen may be inhaled for long periods of time without demonstrable harm, but that concentrations exceeding this are dangerous, irrespective of previous exposure to rich oxygen mixtures.

In the second part of the study experiments were planned to determine whether the inhalation of atmospheres rich in oxygen had a specific inhibitory effect on the development of pulmonary tuberculosis. Acute miliary tuberculosis of the lungs was produced (1) by the intravenous

injection of massive doses of human tubercle bacilli in very young rabbits and (2) by the injection of moderate doses of bovine tubercle bacilli. The concentration of oxygen in most instances was 60 per cent for 22 of 24 hours. In some experiments concentrations of 70 to 85 per cent were employed for periods of two to six hours. No retarding effect on the development of pulmonary tuberculosis was found in any of these experiments. Although an oxygen-treated animal frequently lived longer than the control series death invariably resulted. There were no significant differences in clinical behavior or in the gross and microscopic pathological changes.

In one experiment a more chronic form of pulmonary tuberculosis was produced. A fine suspension of human tubercle bacilli was injected into somewhat larger rabbits than the foregoing series. Repeated doses of this bacillus were given, followed by repeated injections of bovine tubercle bacilli. The oxygen concentration was 60 per cent for 22 of 24 hours. The duration of the experiment was seven and a half months.

Three of the 5 oxygen-treated animals outlived 8 controls. Two of them, alive one and a half months after all the controls had died, succumbed only after they were removed from their oxygen environment. In this experiment the inhalation of 60 per cent oxygen seemed definitely responsible for the increased length of survival of the treated animals. This was evidenced not only by the death ratio of the two groups but by the behavior of the animals themselves. During the last three weeks the two surviving oxygen-treated animals suffered from extreme dyspnea when they were removed from the chamber and were relieved when they were replaced. Both of them died within ten hours after oxygen was discontinued. During their residence in the chamber they attained higher weights than any of the other animals.

The development of tuberculosis, however, was not retarded in the oxygen-treated animals. Repeated X-ray pictures disclosed equally progressive involvement in the two groups. Furthermore, the three animals surviving longest showed the most extensive lesions, notwithstanding their exposure to a rich oxygen atmosphere. Pathological examination likewise revealed a more massive involvement in the animals that lived longest, namely, in the oxygen-treated rabbits. In all the animals large numbers of tubercle bacilli were found in the stained smears of the lung exudate.

Before interpreting these data several facts must be borne in mind. The injection of bovine tubercle bacilli in the doses administered in-

variably causes death in the rabbit, so that a fatal disease was produced in the absence of a specific curative agent. Due to their earlier death three of the control animals did not receive the fifth injection of bovine bacilli. The last injection may be the cause of the more extensive tuberculosis in the three oxygen-treated rabbits which survived longest, particularly as all the control animals died before the effects of this injection could have been fully produced.

An additional reason which may partially explain the increased tuberculosis in the surviving animals is that the inhalation of oxygen prevented death from anoxemia and allowed more time for the extension of the tuberculous process. It was obvious that anoxemia played a large part at the end of the disease and that the relief which oxygen afforded at this time was life-saving.

The increased duration of life of the oxygen-treated animals may be dependent on several factors. In the first place, the prevention of death by anoxemia was obvious from the behavior of the two rabbits that survived longest. Their increased comfort and lessened dyspnea in the oxygen chamber were striking during the last three weeks. It seemed probable also that the inhalation of oxygen decreased the pulmonary ventilation during the period of widespread tuberculosis, and in that way provided more rest as well as prevented the ill effects of lack of oxygen. Poulton (20), in studying dyspnea due to various abnormal conditions in the lung, found that the pulmonary ventilation was usually markedly lowered by the inhalation of oxygen-rich mixtures. In one patient with extensive phthisis the dyspnea was relieved by breathing 40 per cent oxygen. The fact that the tuberculous process itself was not checked indicates the absence of a specific inhibitory effect of the highest concentrations of oxygen compatible with life.

From this investigation there appears to be no rational evidence for the inhalation of oxygen in the treatment of pulmonary tuberculosis unless the condition be attended with dyspnea or cyanosis. Under the latter circumstances oxygen therapy may relieve the dyspnea, decrease the pulmonary ventilation and avert the harmful effects of anoxemia, phenomena which attend the use of oxygen in other acute respiratory disturbances (21).

SUMMARY

1. Experiments were carried out which indicated that the inhalation of atmospheres containing 60 per cent oxygen were without effect on

the general appearance, activity and weight of normal rabbits over periods as long as one to four months. No effect was observed upon their gross and microscopic anatomy for periods as long as one to two months.

2. Attempts were made to increase the resistance of the pulmonary epithelium to atmospheres containing 80 to 85 per cent oxygen by previous exposure to lower concentrations. In all instances, however, exposure to from 80 to 85 per cent oxygen for four to fourteen days resulted in fatal pneumonia characterized by widespread pulmonary edema. In one experiment the inhalation of 70 per cent oxygen for twelve days was followed by pulmonary edema.

3. On the basis of the above experiments the highest concentration of oxygen compatible with safety should be regarded as 60 per cent.

4. Experimental pulmonary tuberculosis was produced by the intravenous injection of human and bovine tubercle bacilli in young rabbits. In the acute, rapidly fatal type of miliary tuberculosis no significant differences in clinical behavior or in gross and microscopic pathological changes resulted from the inhalation of high concentrations of oxygen.

5. In one experiment in which a more chronic form of pulmonary tuberculosis was produced the effect of oxygen treatment was observed over a period of seven and a half months. The duration of life in the majority of the oxygen-treated animals was definitely longer than in the control series. The tuberculous process, however, showed a progressive extension, irrespective of treatment with oxygen.

CONCLUSIONS

The interpretation of the above data leads to the following conclusions:

1. The inhalation of an air-mixture containing 60 per cent oxygen exercises no specific retarding influence on the development of experimental pulmonary tuberculosis.

2. The increased duration of life of the oxygen-treated animals may be explained chiefly through the prevention of death by anoxemia and to a less determinable extent through the restful effect of a diminished pulmonary ventilation.

3. The use of oxygen in pulmonary tuberculosis has no specific curative value. When dyspnea or cyanosis is present the inhalation of oxygen is of benefit as it is in other acute respiratory disturbances, namely, by relieving dyspnea, decreasing pulmonary ventilation and by averting the harmful effects of anoxemia.

We wish to express our thanks to Dr. William C. Clarke in whose department many of the microscopic sections were made and to Dr. Allen K. Krause for his encouraging assistance in the beginning of this investigation.

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STUDIES ON GASTRIC FUNCTION IN PULMONARY TUBERCULOSIS

INCLUDING AN ANALYSIS OF GASTRIC FUNCTION IN 198 CASES OF PULMONARY TUBERCULOSIS.[1]

DAVID PERLA

When we consider that the tuberculosis patient is dependent upon an efficient digestive system as a prime factor in his recovery, it is evident that a careful study of this phase of the disease is of practical value to the patient. The frequency of gastric symptoms in the course of pulmonary tuberculosis is striking. The occurrence of "dyspepsia" is more than coincidental. On the other hand, as Immermann (1) emphasizes, the digestive disturbances are not attributes of tuberculosis but are complications. Fenwick (2) found that dyspepsia is the most frequent symptom of early tuberculosis; 17 per cent of the male and 72 per cent of the female patients had gastric symptoms. Mohler and Funk (3) found them in 64 per cent of 1,000 cases; Hutchinson (4) in 94 per cent of his cases; Landis, in an analysis of 3,007 cases at the Phipps Institute (5), states that 53 per cent had stomach symptoms.

The pathological basis lies in the disturbance of gastric acidity and gastric motility. The underlying factors responsible for the diminished secretion are matters of dispute. Although actual tuberculous ulcers of the stomach have been described by Eppinger (6), Litten (7), Musser (8), and others, their occurrence is certainly very infrequent. Chelmonski (9) found 2 in 86 autopsies of tuberculosis; Fishberg (10) reports only 1 in 128 autopsies at Montefiore Hospital. Gastric ulcer of nonspecific nature occurs with no greater frequency among these patients than among those of any group. The dyspepsia has been attributed to toxemia resulting from the active tuberculous process, as maintained by Fenwick (2), and to pyrexia by Croner (11) and Hilderbrandt (12), although it has been repeatedly demonstrated "that one of the characteristics of the anorexia of phthisis is that unlike the appetite of other diseases it is independent of the fever in many cases" (Fishberg (10)).

[1] From the Tuberculosis Service of Montefiore Sanatorium, Bedford Hills, New York.

Ritter and Hirsch (13), Edinger (14), and Kredel (15) attribute it to the anemia which may accompany the early stages of tuberculosis, and which results in a generalized ischemia of the digestive tract. Chelmonski (9) found in half of his autopsied cases marked anemia of the gastric mucosa. It has been found, however, by Janowsky (16) and others that many cases without anemia present gastric disturbances. We have found this to be true. In advanced cases the general atony of the general musculature, both smooth and striate, results in gastrectasis, as was shown by Fenwick in 100 autopsies (cited by Fishberg (10)).

Aside from the clinical study of the symptomatology, analyses of the gastric contents after test-meals have been done by a great many workers, and the reported results are a maze of contradictory evidence. The methods used varied, and the standards of normality differed. There is often no attempt to differentiate the stages of the disease or to determine the presence of activity. The literature may be summed up as follows: Brieger (17) and Klemperer (18) find the motor power of the stomach weakened in proportion to the chemical change, and with Robin and dePasquier (19) they observe a diminution of the hydrochloric acid in proportion to the extent of the disease. Shetty (20) concludes that the dyspepsia is in no way dependent upon gastric catarrh, since there is no change in the secretion of acid, time of digestion or motility of the stomach. Immermann, (1), Einhorn (21) and Croner (11) find no diminution in motor function. All the above-mentioned authors but Brieger (17) report hyperacidity in the early stages. Croner (11) and Immermann (1) find the acidity usually normal, while Rosenthal (22), Bordoni (23), and Chelmonski (9) often note an achlorhydria. Munson (24) states that the motor power and acidity are undisturbed in the early stages, that often hypochlorhydria and achlorhydria may occur at different times in the same individual, and that the alteration of acidity is not a result of fever. Generally, in the later cases there is hypoacidity although hypoacidity may occur in all groups. Mohler and Funk (3), using the fractional method of Rehfuss, find hyperacidity rare in the early stages, and state that pulmonary tuberculosis causes a progressive diminution in the secretory function and motility of the stomach.

Methods and standards: We have analyzed the gastric contents of 198 consecutive patients of the Bedford Sanatorium for Tuberculosis

(Bedford Hills, New York). Of these, 92 were males and 106 females. We employed the large stomach tube with an Ewald evacuator. The contents of the morning fasting stomach were first examined. A test-meal of one large slice of toast with 500 cc. of water was given, and from forty-five minutes to one hour afterward the contents were again removed. Ten cubic centimetres of the filtered juice was titrated against a n/10 sodium-hydroxide solution, using three drops of Töpfer's reagent (phenyldimethylamidoazobenzol) in the determination of the free hydrochloric acid, and 6 drops of phenolphthalein reagent in the determination of the total acidity. The standard of normality in terms of n/10 sodium hydroxide per 100 cc. of gastric juice was taken as 20 to 40 cc. for free hydrochloric acid and 40 to 60 cc. for the total acidity. Hyperacidity was determined by higher amounts than these; moderate hypoacidity by a hydrochloric-acid content of 11 to 20 cc.; and marked hypoacidity by a hydrochloric-acid content of less than 11 cc. with a total acidity of less than 25 cc.

The single specimen at the end of three-quarters of an hour to an hour, together with the specimen of the fasting contents, gives a fair idea of the gastric function in most cases. After careful observation under the fluoroscope in many cases, I found that the tube did not curl up as has been maintained by Harmer and Dodd (25) and by Rehfuss (26). There is no particular advantage in the use of the fractional test-meal of Rehfuss. The work of Gorham (27), Wheelon (28), White (29) and Kopeloff (30) have adequately demonstrated that extractions of gastric juice, removed either in rapid succession or simultaneously by tubes of different lengths, have different acid contents. It is therefore more advisable to remove the entire contents after the Ewald test-meal.

Results: Seventy-five per cent had a definite diminution of the hydrochloric-acid secretion, 10 per cent had no free hydrochloric acid, 27 per cent had a marked hypochlorhydria, and 37 per cent a moderate hypochlorhydria. About 25 per cent secreted normal or more than normal amounts of hydrochloric acid. Only 2 per cent had a hyperchlorhydria. Of 198 cases analyzed the results were about the same in the two sexes.

A series of blood counts in 50 consecutive patients eliminated anemia as an important contributing factor in the hypoacidity found in tuberculosis. Very few cases had more than a slight anemia.

Relation of anatomical stage of disease to acidity: Sixty-nine per cent of cases with normal gastric function, however, were found in patients with cavitation in one or both lungs. Of the 5 cases with hyperchlorhydria 4 were in patients with far-advanced lesions with cavitation. Of the cases in stage 1A (Turban classification) 50 per cent showed a definite diminution in the hydrochloric-acid secretion. Of the cases in stage 2A, 67 per cent showed a hypoacidity. More than half of these had a free-acid content of less than 10 (expressed in cc. n/10 NaOH per 100 cc. of gastric content); 17 per cent had a complete anacidity. In stages 3A and 3B, 80 and 75 per cent respectively of these groups had a definite hypochlorhydria. In stage 3B, 46 per cent were below 10, with a complete absence of free acid in 14 per cent. The extent therefore of the anatomical pulmonary lesion has little to do *per se* with impaired gastric function.

Relation of acitivity (with fever) to acidity: The presence of fever is not necessarily associated with diminished hydrochloric secretion (Munson (24)), as is generally the case in acute infections. However, 75 per cent showed a diminished hydrochloric-acid secretion. In 46 per cent the acid content was below 10, while 25 per cent of the cases with fever and 25 per cent of those without fever had a normal acid content. Two active cases had a hyperchlorhydria. The absence of hydrochloric acid was more frequent in cases with constitutional symptoms (14 per cent) than in those without (8 per cent).

Relation of gastrointestinal symptoms to gastric acidity: The various complaints referable to functional disturbances of the gastrointestinal system are very numerous, but become serious only when they interfere with the assimilating capacity of the patient. Many patients complain of transient disturbances of digestion, but if the complaints are persistent they are, in almost every case, attributable to disturbances of gastric function in the absence of evidence of tuberculous enteritis. Eighty per cent of all our patients had symptoms referable to the gastrointestinal tract, and, of these, 76 per cent had a decrease in the hydrochloric secretion. The more prominent complaints were constipation (25 per cent), distention (19 per cent), feeling of fulness after meals and anorexia (19 per cent), belching and heartburn (13 per cent), sour eructations (9 per cent), and cramps (7 per cent). The more prominent symptoms associated with hypoacidity were distention, constipation,

anorexia, and sensation of fulness after meals. Sour eructations occurred as frequently with marked hypoacidity as with hyperacidity. Heartburn was common in cases of hypoacidity. It is maintained by some writers that the gastric contents after the usual early morning test-meal may show a hypoacidity, but that removal of the contents one or two hours after a full meal may reveal a hyperacidity and thus account for the heartburn and sour eructations. In several cases we could not confirm this. Heartburn, however, is not a symptom of hyperacidity, but is due to regurgitation of the stomach contents into the esophagus. Sour eructations generally result from acid substances produced by the decomposition of undigested foods, and are therefore more likely to occur with diminished hydrochloric acid than with an excess of it. They occur several hours after meals. The abundant use of sodium bicarbonate gives at times a temporary relief by neutralizing the acid thus regurgitated. Its promiscuous use in such cases is to be condemned until the nature of the dyspepsia is ascertained. The few cases of tuberculous enteritis that we had in our series showed hypermotility with hypoacidity of the stomach. All cases with emetic cough had hypoacidity. Of the 21 cases of anacidity 7, or $33\frac{1}{3}$ per cent, had no gastric symptoms. It is, of course, true that the absence of hydrochloric acid does not necessarily lead to gastric complaints. As was pointed out by Hutchinson (4), some of the patients show a definite distaste for fats, and diminution in this part of the diet affords relief. It cannot be too strongly emphasized that vague gastric complaints or occasional attacks of cramps with constipation may be the early manifestations of tuberculous enteritis.

Motility: If more than 50 cc. of the gastric contents is obtainable after the test-meal the case is considered one of hypomotility. If no contents are obtainable the stomach is hypermotile. All the cases of hypermotility were associated with hypoacidity. The cases of marked hypermotility showed other evidences of organic involvement of the intestine, such as tuberculous enteritis. Hypomotility is generally associated with hyperacidity; that is, large amounts of gastric contents were generally present in cases of hyperacidity. All the cases of hyperacidity were associated with hypomotility, whereas of the 110 cases of hypoacidity only 15 were associated with hypomotility.

Fluroscopic studies of the position of the stomach: Although patients with ptosis of any degree may show no secretory disturbance, very marked gastroptosis is more often associated with diminished acidity. In order to determine whether this played a factor in the cases of tuberculosis with diminished acidity, careful studies of the position of the stomach after a barium meal were made.

The normal stomach varies greatly in size and position, motility and mobility, and varies with the bodily form of the individual. In persons of gastric or sthenic habitus it is high and small, and lies horizontally; in those of asthenic habitus it is long and relaxed, and the pylorus may lie to the left of the midline. The form of the stomach may be horn-

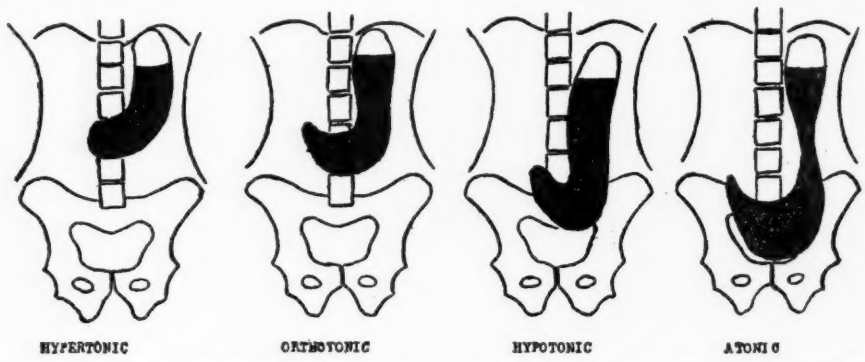

FIG. 1. DIAGRAMS ILLUSTRATING VARIATIONS IN THE SHAPE AND POSITION OF THE STOMACH

These four figures are recognized as types of stomach which, while varying considerably, show no marked evidence of gross disease distinguishing them from the forms in which organic disease is present. (From Knox, *Radiography and radiotherapeutics*, London, 1917, vol. i.)

shaped or J-shaped. According to Schlesinger (31) the form of the stomach depends on its tonicity. A definite degree of *tonus* results in a characteristic appearance. Furthermore, the tonicity of the abdominal musculature will influence the position of the stomach. Groedel (32), in a careful analysis of the subject, found that the J-shaped stomach (*Hakenform* of Rieder or the *Siphonform* of Groedel) was the form most often associated with normal function. On the basis of Schlesinger's classification of the tonicity of the stomach, Knox (33) uses the standard illustrated in figure 1 to indicate the variations in the position and shape of the stomach. Groedel's normal would correspond to the orthotonic type, J-shaped and with both borders above the crest of the ilium. The hypotonic and atonic forms are both definitely ptotic. Normal gastric

function may be associated with any of the forms, although least commonly with the atonic type.

We have tabulated the results of a study of the position and form of the stomach in 100 consecutive cases of pulmonary tuberculosis taken from our 198 gastric studies reported in this paper. Measurements were taken of the exact position of the borders of the stomach in relation to the intercristal line and the umbilicus. The position of the pylorus in relation to the midline of the abdomen was noted. An analysis of this material shows that two-thirds of the cases fell into the orthotonic group and about one-third were hypotonic. Only two cases were of the atonic type. Of the orthotonic group, almost half had marked hypochlorhydria, 25 per cent had moderate hypochlorhydria, and only 14 per cent had normal gastric function. About one-third of the entire series had definite ptosis of the stomach; two-thirds of these were associated with diminished gastric function, most of them with marked hypochlorhydria. On the other hand, one-third showed no abnormality of gastric secretion. It is interesting that of the cases with normal secretion more than half showed moderate ptosis of the stomach. About half of the ptotic group were definitely of the asthenic habitus. About two-thirds of all the cases with gastrointestinal symptoms had a normally placed stomach, and one-third had gastroptosis. One-third of those without symptoms also had gastroptosis. Although 100 cases are not a sufficient number from which to draw any definite conclusions, it is obvious that a normal J-shaped orthotonic type of stomach is found in most cases (two-thirds) of tuberculosis uncomplicated by intestinal lesions. (This excludes patients in the very end-stage, in which marked ptosis may be associated with general muscular atony.) Further, although ptosis of the stomach may be expected in every third case of tuberculosis, it is not necessarily associated with impaired gastric function, although this is the more common finding. In half of the cases with ptosis the dropped position of the stomach is associated with the loss of abdominal muscular tone. Among sanatorium patients only one in six has a definite asthenic habitus with gastroptosis. Of the cases with normally placed stomach three of every four show some impairment of gastric function, but of those cases that have normal gastric secretion half have gastroptosis of a mild type. It is evident, therefore, that the position of the stomach does not contribute to the impaired gastric function found so often in cases of pulmonary tuberculosis.

Treatment of hypoacidity The beneficial results that may be obtained by the administration of dilute hydrochloric acid in those cases with gastric symptoms arising from a diminished secretion is striking. An analysis of the stomach contents in every case with distressing gastric complaints, particularly when these interfere with the nutrition of the patient and hinder his progress, is imperative. In many cases with dininished acidity we have observed immediate symptomatic relief of marked anorexia, distention and heartburn after the administration of 30 minims of dilute hydrochloric acid with meals, even without change in diet. This obvious procedure is, however, often overlooked. A bland diet with avoidance of gas-producing foods, such as coarse green vegetables, pastries and fancy salads (as recommended for flatulence by Alvarez in *Oxford Medicine*) may alleviate the distention. Helping the patient get adequate nourishment is a very important factor in aiding recovery. It must be emphasized that tuberculous enteritis may begin with vague gastrointestinal complaints, occasional cramps with constipation, or indefinite pains in the abdomen. If the symptoms persist a gastrointestinal series will aid in confirming the diagnosis.

CONCLUSIONS

1. Pulmonary tuberculosis is generally accompanied by a diminution of acidity of the gastric juice.

2. The decrease in hydrochloric acid bears no definite relation to the anatomical extent of the disease, although anacidity occurs more frequently in active bilateral far-advanced lesions.

3. Although the presence of fever is not necessarily accompanied by a diminished hydrochloric-acid secretion, marked hypochlorhydria is more frequent in cases with constitutional symptoms than in those free from them.

4. Heartburn and sour eructations are far more frequently associated with hypochlorhydria than with hyperchlorhydria. Distention, anorexia, belching and constipation are, however, the more common symptoms associated with hypoacidity.

5. All cases of emetic cough show marked hypoacidity.

6. The absence of free hydrochloric acid may occur in cases without any gastrointestinal complaints.

7. A normal J-shaped orthotonic type of stomach is found in the majority of cases of pulmonary tuberculosis uncomplicated by intestinal lesions. In cases associated with gastroptosis impairment of gastric

function is not necessarily present. The position of the stomach does not contribute to the hypochlorhydria so often found in cases of pulmonary tuberculosis.

8. In pulmonary tuberculosis associated with hypoacidity large doses of dilute hydrochloric acid affords, in the presence of disturbing digestive complaints, immediate symptomatic relief, and contributes to the general improvement of the patient.

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CLINICAL EXPERIENCES INDICATING THE IMPORTANCE OF TIMELY RADICAL LOCAL REST (THORACOPLASTY) IN PULMONARY TUBERCULOSIS[1]

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The modern sanatorium is rapidly drifting into an institution that requires all the equipment and accommodations of the general hospital (1), as well as those of the sanatorium, and possessing such facilities for the prolonged residence of the patient as recreational and educational features, beside some special facilities such as those for heliotherapy, etc. As publicity spreads information on hygienic and sanitary measures, including dietetic and other methods of treating early cases of pulmonary tuberculosis, these are being cared for in the home, and it is only the patient who has gone beyond the realm of conservative therapy that enters the sanatorium, requiring at this stage distinct radical methods of treatment in most instances. The sanatorium at present thus receives predominantly two groups of cases, the one a large group of the hopeless type, with bilateral involvement complicated perhaps by extrapulmonary lesions, and the second group with pulmonary lesions chiefly unilateral in type, arriving in serious condition but still amenable to radical therapy. It is in the differentiation of the two groups that the sanatorium physician is placed on his mettle. As time passes, a survey of past errors in judgment serves to lay out his future course, and gradually increases the efficiency of the medical service in returning to active life an ever-increasing number of even the apparently hopeless cases of the past decade. The rapid perfection of technique in thoracic surgery and its more general application during the past five years as a supplement to artificial pneumothorax have been material aids in enlarging the list of restored cases, in spite of their advanced condition upon entrance to the sanatorium. It is common knowledge that to-day the incipient case in the sanatorium is rare.

Since the greatest opportunity for radical treatment remains with the

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cases which are to be grouped as mainly unilateral, and for which no other course except radical procedure lies open, such as cases of spontaneous pneumothorax, it is the purpose of this paper to review a number of cases in which artificial pneumothorax, phrenicotomy and thoracoplasty were not applied, and the usual fatal termination ensued, and a number of almost identical cases in which these methods proved successful and by means of which the patient was restored to an active life.

It is still with reluctance that some tuberculosis specialists utilize artificial pneumothorax, and many times its value is even questioned in some medical circles. This pessimism has probably been engendered by the occasional accident coincident to its use (2), and probably in greater proportion by a poor selection of cases, inadequate technique or inexperience of the operator. In properly regulated surroundings and with careful selection of cases, artificial pneumothorax has proved of inestimable value, as is borne out in international literature. A few successful cases with interesting features will serve to illustrate the efficacy of artificial pneumothorax in carefully selected cases.

Case 1: An Italian girl, 17 years of age, was referred to the Outpatient Department of the National Jewish Hospital after a residence of several months without benefit in a local sanatorium. There was a family history of tuberculosis, and personal onset of pulmonary tuberculosis with brisk hemoptysis about seven months prior to physical examination, which disclosed a far-advanced condition with signs of cavitation in the upper lobe of the left lung and scattered râles to the level of the sixth rib in the midclavicular line anteriorly. The right lung disclosed only an occasional râle in the apex upon deep

Description of figures 1 to 6

FIG. 1. Roentgenograph of case 2, taken three weeks before the initiation of artificial pneumothorax and showing predominantly left monolateral tuberculous involvement.

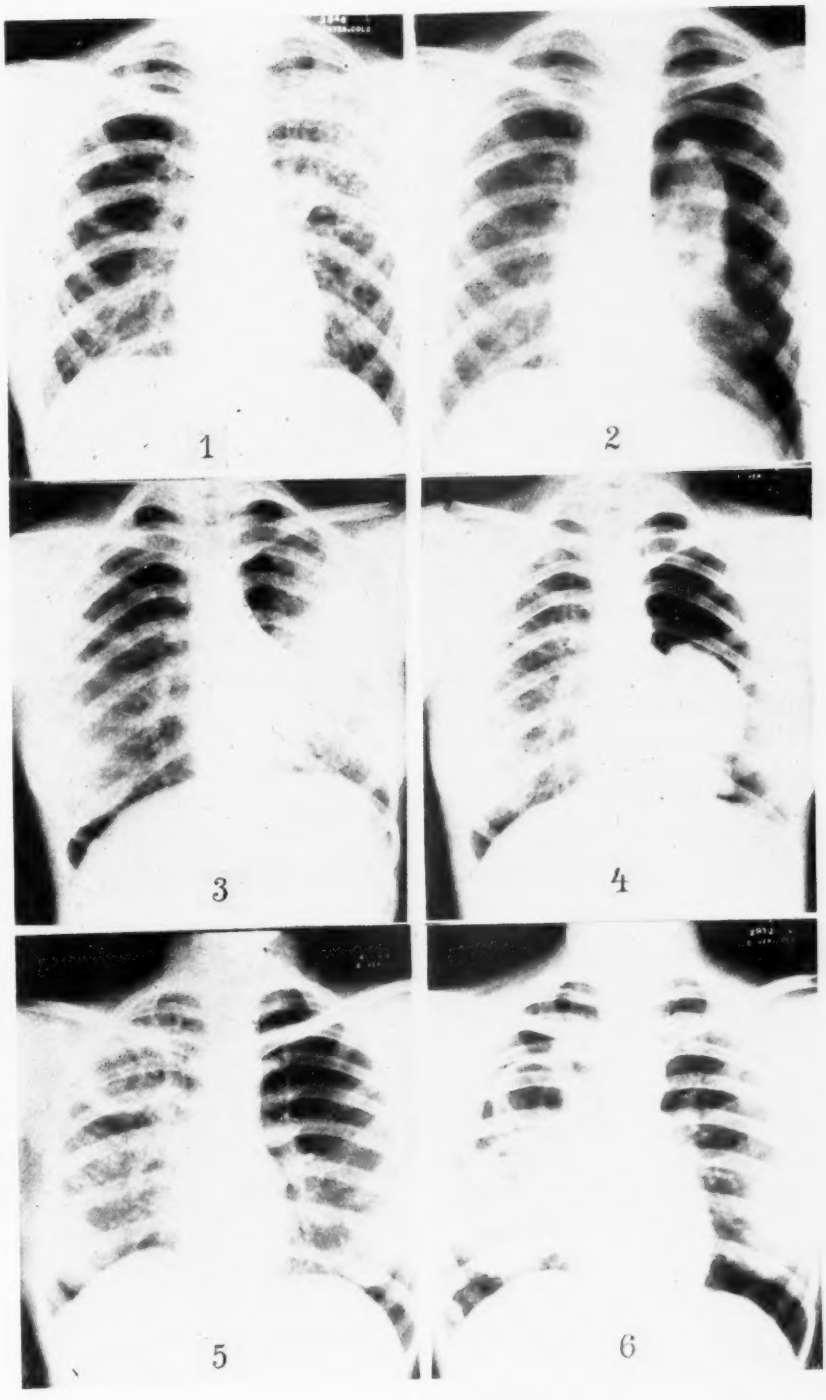
FIG. 2. Roentgenograph of case 2, taken two months after initiation of artificial pneumothorax and showing excellent collapse of the entire left involved lung with resultant striking improvement in the patient's condition.

FIG. 3. Roentgenograph of case 3, taken two weeks before the initiation of artificial pneumothorax, showing left monolateral tuberculous involvement.

FIG. 4. Roentgenograph of case 3, taken two months after initiation of artificial pneumothorax, showing excellent collapse of the entire left lung, which resulted in a gradual but complete subsidence of all symptoms, with complete recovery within eight months.

FIG. 5. Roentgenograph of case 4, taken two months prior to the initiation of artificial pneumothorax, showing predominant right monolateral tuberculous pulmonary involvement.

FIG. 6. Roentgenograph of case 4, taken three weeks after the initiation of artificial pneumothorax, showing failure to collapse the involved right lung, with persistent patency of the cavities from which, in spite of 7 months' artificial pneumothorax, there occurred a single brisk hemoptysis with immediate fatal termination.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inspiration, and increased vocal and tactile fremitus in the same region. The afternoon temperature for the past month ranged from 99.5° to 100.5°. The sputum was positive for tubercle bacilli. Artificial pneumothorax on the left side was instituted three days after examination, the decision resting upon the slight involvement of the right lung clinically and roentgenologically, the presence of cavitation in the left upper lobe, the pyrexia, loss of weight, positive sputum and general unsatisfactory progress of the case, and all evidence available pointed to the possibility of obtaining a good collapse with a free pleural space. The collapse was instituted by an initial injection of 400 cc. of air, and a final reading of 0, −2 was attained. Injections were given every four days for the first two weeks, an average of 300 cc. being administered; and the collapse was subsequently maintained by weekly injections for two months. For the last two months only semimonthly injections were required to maintain the collapse. Roentgenological examination disclosed a good compression of the entire left lung and especially of the cavity in the upper lobe. There was a rapid subsidence of the fever, cough and expectoration, and the general condition of the patient is good four months after the institution of artificial pneumothorax.

Case 2: Clerk, 21 years of age, entered the hospital with a history of illness dating three months, a daily temperature of 101° to 103.5°, cough and expectoration, and hemoptysis two months prior to admission. The patient had had measles two years and malaria eighteen months before admission. Physical examination revealed an undernourished, bedridden man, 6 feet tall, and weighing 119 pounds, a slight exophthalmus and congested larynx. There was increased whispered and vocal fremitus, with bronchial breathing and moist râles to the fourth rib on the left side anteriorly and posteriorly, while the right lung was practically negative; temperature on admission 103°, pulse 120. Roentgenological examination disclosed a far-advanced acute exudative pulmonary tuberculosis of the left lung, with an annular shadow near the hilum and a few exudative areas in the upper lobe of the right lung. Repeated microscopic examinations of the sputum failed to disclose tubercle bacilli and guinea-pig inoculation was negative. The rapid progress of the case with chiefly monolateral involvement, the annular shadow and the history of hemoptysis determined the use of artificial pneumothorax, which was instituted three weeks after admission. A good compression was obtained, and an average of 400 cc. was administered weekly. Within six weeks after pneumothorax was instituted the temperature became normal, the pulse dropped from 120 to 80, the appetite improved, and weight increased 8 pounds. The patient was discharged upon request as an arrested case, symptomless, and capable of work four months after the initiation of artificial pneumothorax.

Case 3: Girl, 18 years of age, weighed 100 pounds and was 5 feet tall. Family history was negative. In 1918 she had an influenza attack lasting two weeks, and in 1923 an appendicectomy was performed, to be followed two months later by influenza which confined her to bed for two weeks and from which she never quite recovered but had persistent morning sputum of about 1 ounce daily. She had no hemoptysis; but she had night-sweats, and tubercle bacilli were found six weeks prior to entrance into the sanatorium. Upon admission, physical examination revealed far-advanced pulmonary tuberculosis, with impaired resonance down to the ninth rib anteriorly and posteriorly on the left side, and moist râles in abundance but no signs of cavity. The right lung was practically negative. Roentgenologically, there was found a marked inflammatory and fibrous infiltration throughout the entire left lung, with pleuropericardial adhesions and peribronchial thickening, as well as some enlarged lymph nodes in the right lung. Her temperature, pulse and respiration on admission were 100°, 90 and 20. The unilateral involvement, in a young girl with disease of apparently short duration, suggested the probability of instituting a successful artificial pneumothorax. Air was injected after about three weeks of observation and a good collapse was obtained. The temperature, pulse and respiration returned to normal within eight months, there was a gradual but steady increase in weight up to 117 pounds, cough and expectoration diminished, and the sputum was negative for tubercle bacilli persistently for four months prior to discharge. Under pneumothorax treatment the patient was still well one year after discharge.

After all, the three above-cited cases in which successfully artificial pneumothorax produced striking therapeutic results would not be exceptional in the experiences which many physicians have had in the employment of this measure of local pulmonary rest, but still they stand in marked contrast to the cases in which this procedure was not used because of an ultraconservative attitude or to cases in which it was impossible to institute it sufficiently successfully to accomplish the desired results. A case of the latter type with interesting features is depicted in the following report:

Case 4: An American girl, single, 23 years of age, was 5 feet, 4 inches tall, weighed 106 pounds, had a negative family history, except that one brother had tuberculosis, and a personal history of amoebic dysentery ten years and acute influenza three months prior to admission to the National Jewish Hospital. Her health had been good prior to the attack of influenza. Upon examination, the patient complained of progressive loss of weight, persistent productive cough, and afternoon elevation of temperature. Physical examination revealed far-advanced pulmonary tuberculosis limited to the right lung, with

signs of cavernous breathing in the upper and middle lobes, and moist râles elicited with normal respiration. The left lung was practically negative. The patient was hoarse, and laryngeal examination disclosed injected arytenoids. Roentgenological examination revealed a large annular shadow in the vicinity of the right clavicle, extending from the inferior border of the first rib to the superior border of the third rib on the right side. There was a moderate amount of inflammatory and fibrous infiltration of the rest of the right lung except at the costophrenic angle which remained clear. The left lung was negative except for slight general peribronchial thickening. The sputum was laden with tubercle bacilli, urine negative, erythrocytes 4,080,000, hemoglobin 80 per cent, and leucocytes 7,000. The temperature, pulse and respiration on admission were 101.4°, 126 and 24. After the ordinary general rest and dietetic treatment, for two months, with no improvement in symptoms and physical signs, artificial pneumothorax was instituted, and continued weekly by giving 300 to 350 cc. of air, which gave a negative final reading. Fluoroscopic and roentgenological examination disclosed that a complete collapse could not be attained because of adhesions, and the cavity remained patent. Artificial pneumothorax was continued over three months with no marked improvement in the laryngeal condition or cough, and only a slight daily reduction of temperature. Seven months after admission a severe coughing spell was followed by a brisk hemoptysis, the only one recorded, which terminated in immediate death. Briefly, the autopsy findings were: Dense fibrous adhesions extending from the upper and middle lobes of the right lung to the posterior and lateral walls of the chest, which prevented the collapse of multilocular cavities in the upper and middle lobes. One of the cavities in the middle lobe contained a large blood-clot. The remainder of the right lung contained numerous caseous tubercles of varying sizes. There was a small amount of straw-colored fluid in the right pleural space. The left lung was not tuberculous. The remaining organs of the body were negative.

This case, although to all intents and purposes identical with the first three cases of successful treatment with artificial pneumothorax, indicates that even this procedure can be applied with a conservatism toward more radical methods that runs the danger of overlooking a life-saving opportunity. When, after a short trial, a successful collapse had not been accomplished, especially with a monolateral involvement, the more radical surgical procedures should have been resorted to. This case is a striking example of a therapy applied without success with as little foresight as though phrenicotomy and thoracoplasty were not available.

The next case to be presented bears out the importance of discontinuing a procedure which is not achieving the results desired, and of utilizing a more drastic method of rest therapy.

Case 5: A young male accountant, 21 years of age, with a history of family tuberculosis and previous personal sanatorium treatment, with slight hemoptysis, entered the National Jewish Hospital with cough, blood-tinged expectoration, temperature, pulse and respiration, 99.4°, 85 and 20, and loss of weight. Physical examination revealed far-advanced pulmonary tuberculosis confined mainly to the right side, with signs of cavity in the upper lobe of the right lung, and moist râles and dulness in the upper and middle lobes. The apex of the left lung showed increased vocal and whispered fremitus and dry râles. The roentgenological findings were marked consolidation, with rarefied annular shadows in the right upper lobe, and fibrous and inflammatory mottling throughout the remainder of the right lung. There were marked bilateral fibrous infiltration and enlargement of the hilum, and the mediastinum was pulled to the right in the upper portion. The left lung revealed some inflammatory and fibrous infiltrations throughout the upper lobe with scattered infiltrations in the lower lobe. There were adhesions to the diaphragm on both sides. Numerous tubercle bacilli were found in the sputum. The blood and urinary findings were normal. The patient was placed at absolute rest because of his blood-streaked sputum. During eighteen months' residence in the sanatorium the case was not unusual except for an occasional hemoptysis that was controlled by rest in bed and ordinary hemostatic therapy, such as thromboplastin, 10 per cent sodium chloride and calcium chloride. After eighteen months the patient was discharged, with positive sputum and slightly improved, to a neighboring convalescent sanatorium, where he remained for six months, when he was readmitted to the National Jewish Hospital as an emergency case because of a brisk and uncontrollable hemorrhage. His clinical findings had changed very little from those of his first admission. Absolute bed-rest and the usual medical treatment for hemorrhage proved unsuccessful, as did several attempts to institute artificial pneumothorax owing to a lack of a free pleural space. The first stage of a thoracoplasty was performed three weeks after the second admission, when the eleventh, tenth and ninth ribs were removed under local anaesthesia. There occurred during the third night after operation a brisk hemoptysis which subsided. Subsequently, the thoracoplasty was completed in two more stages with the removal of parts of all the ribs, including the first, on the right side. There was gradual and steady improvement in the patient's condition, a good collapse having been obtained, and at the present writing the patient has a persistently negative sputum, expectoration is reduced markedly and he is seeking a position. The noncollapsed lung has cleared up, and reveals little or no findings on physical examination; thus bearing out the earlier contentions of Simon (3) and of Schill (4) as to the influence of artificial pneumothorax on the contralateral lung.

This case is a striking example of one in which general rest (immobilization) and artificial pneumothorax failed to accomplish the desired arrest, and during all the period of several years of treatment the patient was in constant danger of a fatal hemoptysis such as occurred in case 4. Thoracoplasty achieved the desired collapse of the cavity in the right lung, and with it abolished the source of hemorrhage and tubercle bacilli in the sputum. The patient enjoys an active life at present, ten months after the final stage of his thoracoplasty.

Spontaneous pneumothorax, occurring during the course of pulmonary tuberculosis, either without or during treatment with artificial pneumothorax, offers to the tuberculosis physician in the majority of cases a very perplexing problem, so far as prognosis and treatment are concerned (5). The mortality is high (80 per cent (6)), and treatment is symptomatic (7). Two cases with interesting features are reported below, one treated conservatively and contributing to the present high mortality which Kahn deplores, and the other, a more severe case to all intents and purposes, treated radically with phrenicotomy and thoracoplasty with recovery and return to an active life.

Case 6: Draughtsman, 23 years of age, mother died of tuberculosis. The patient suffered an attack of typhoid fever seven months prior to admission to the National Jewish Hospital, following which there were persistent cough with expectoration and loss of weight. Physical examination revealed a well-developed, well-nourished man, 5 feet, 11 inches tall and weighing 159 pounds. Respiratory mobility was decreased on the left side, the apex-beat visible in the fifth interspace in the midclavicular line, tactile fremitus diminished on the left side down to the sixth interspace in the midaxillary line, and tympany present anteriorly and posteriorly in the apex and merging to dulness and flatness to the lower border of the fourth rib with distant breath-sounds and faint vocal fremitus. There were occasional moist râles in the apex of the left lung with cavernous breath-sounds. A succussion splash was marked. The right side revealed only increased vocal and whispered fremitus in the apex and an occasional dry râle on coughing. Roentgenological examination disclosed far-advanced pulmonary tuberculosis of the left side, with a spontaneous pneumothorax having two distinct fluid levels (fig. 9), one at the fifth interspace and the other at the seventh interspace. The cardiac shadow was displaced to the right. There was moderate infiltration of the apex of the right lung. The sputum was positive for tubercle bacilli, hemoglobin 70 per cent, erythrocytes 4,260,000, leucocytes 11,800 with neutrophiles 76 per cent, and urinalysis negative. On admission the temperature, pulse and respiration were 99.5°, 85 and 24. Owing to the increase of hyperpnoea and dyspnoea,

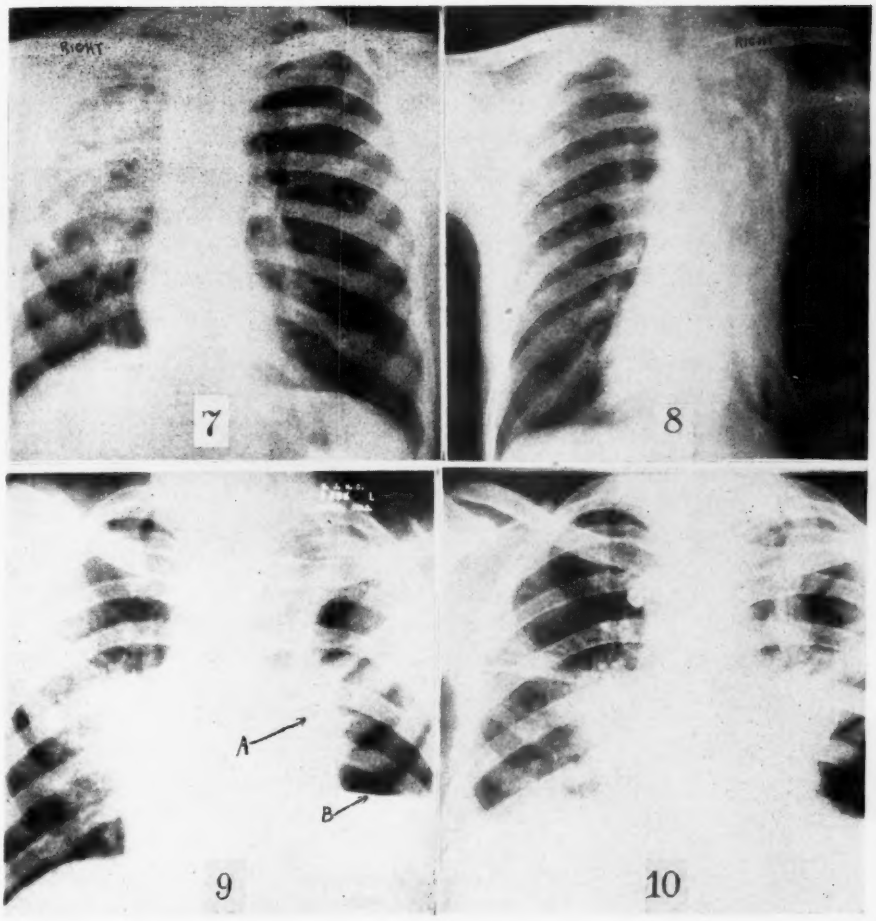


FIG. 7. Roentgenograph of case 5, taken two weeks prior to thoracoplasty and showing right monolateral tuberculous pulmonary involvement.

FIG. 8. Roentgenograph of case 5, taken ten months after a complete right-sided thoracoplasty following numerous mild and a single uncontrollable brisk hemoptysis. Attempts to induce an artificial pneumothorax were unsuccessful. There resulted a complete control of the hemorrhage and a gradual and steady improvement in the patient's condition with complete absence of symptoms.

FIG. 9. Roentgenograph of case 6, taken two days after admission and showing the two fluid levels *A* and *B* and spontaneous pneumothorax on the left side.

FIG. 10. Roentgenograph of case 6, taken three months after admission and after tube-drainage was instituted, which, however, had no influence upon the fluid level in the upper pocket. At this time the costophrenic angle on the right side was obscured. The patient died five months after admission, in spite of drainage of the main left pleural space.

accompanied by a rise in temperature to 101° and acceleration of pulse to 120, thoracentesis was performed two days after admission, when 1000 cc. of pale yellow serous fluid containing tubercle bacilli was removed and partially replaced by air. The aspirations of fluid were resorted to at weekly intervals to relieve the dyspnoea, and the fluid gradually became more turbid. A drainage tube had to be inserted finally, to obviate the necessity for repeated needle drainage and to provide a means of washing the pleura with Dakin's solution. However, the toxic symptoms did not abate, but physical examination of the contralateral (right) lung two and one-half months after admission showed an advance of the disease, as evidenced by the presence of moist râles elicited on normal respiration down to the fifth interspace. Signs of pericarditis with effusion appeared four months after admission, with moderate edema of the face and extremities, and suggesting cardiorenal impairment. There occurred at this time positive urochromogen and diazo reactions. The patient died five months after admission. At autopsy there was found a thickened pleura on the left side, studded with tubercles and with dense adhesions especially at the level of the fourth rib. The upper lobe of the left lung contained numerous communicating cavities, and one of these connected with the upper pleural pocket by means of a bronchial fistula at the level of the second rib. The fluid in this pocket gave the upper line of fluid accumulation in the X-ray at the level of the fifth rib. There was another large bronchial fistula, between the upper and lower lobes, draining into the main pleural space, which was responsible for the accumulation of pus giving the lower fluid level. The upper and smaller pleural empyema pocket was separated from the lower main empyema by means of dense fibrous adhesions extending from

Description of figures 11 to 15

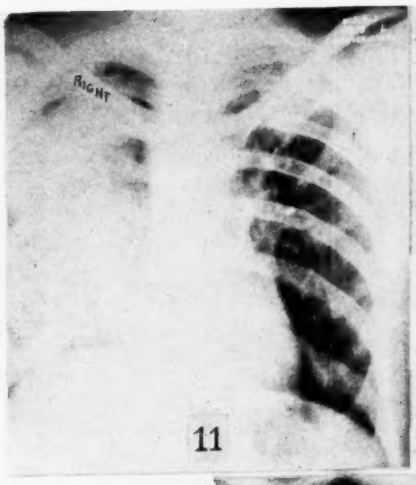
FIG. 11. Roentgenograph of case 7, one week after first admission and showing predominant right monolateral tuberculous pulmonary involvement.

FIG. 12. Roentgenograph of case 7, fourteen months after first admission, and three months after reinstitution of artificial pneumothorax resulting in good collapse of the right lung with subsequent return to an active life under continuous artificial pneumothorax.

FIG. 13. Roentgenograph of case 7, three years after first admission, eighteen months after first discharge, and the second day of second admission with a spontaneous pneumothorax superimposed on an artificial pneumothorax. Note accumulation of pleural fluid *E*.

FIG. 14. Horizontal roentgenograph of case 7, after diagnostic pleural injection of sodium-iodide solution to locate the bronchial fistula, five months after second admission, and while daily pleural washings through a drainage tube were necessary to prevent accumulation of pleural fluid and toxic symptoms. A small elevation is noted opposite the sixth rib (arrow), which was believed to be the bronchial fistula. The picture was taken during the inspiratory phase.

FIG. 15. Roentgenograph of case 7, thirteen months after second admission and about nine months after right-sided phrenicotomy and partial thoracoplasty (eleventh, tenth, ninth and eighth ribs), which resulted in improvement and restoration to an active professional life five months after operation.



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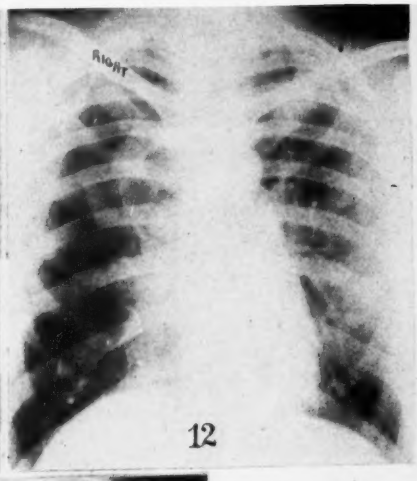

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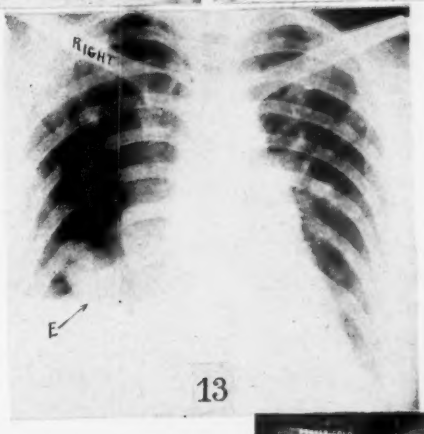

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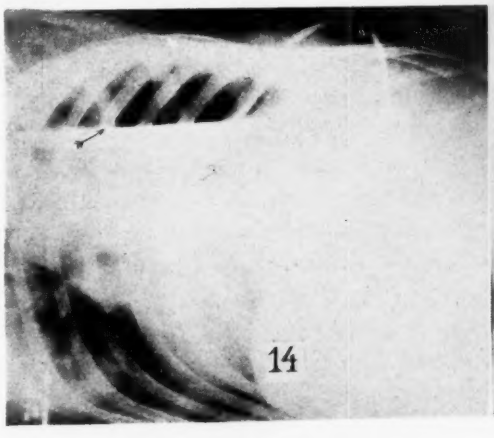

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the fourth rib. The lower main pleural empyema had been drained by a tube which did not enter or affect the upper-level empyema. The upper lobe of the right lung was densely adherent, and there was caseous pneumonia of the upper lobe. The heart showed a general ventricular hypertrophy, and there was an excessive amount of a slightly turbid pericardial effusion.

The following case handled successfully is presented for its value in shedding light on the treatment and outcome of the foregoing case:

Case 7: The observation of this case, a physician 31 years of age, extended over a period of four years, and only the more interesting phases of the case after second admission will be detailed here. The patient, of nontuberculous parentage, was admitted for the first time to the National Jewish Hospital in 1921 in a markedly toxic condition, weighing 135 pounds and with height 5 feet, 11 inches. Physical and roentgenological examinations revealed far-advanced tuberculosis of the right lung with practically no involvement of the left. Right artificial pneumothorax was instituted one week after admission, but the unfavorable effect on the contralateral lung made discontinuance of treatment seem expedient after one month, when the patient was placed on immobilization treatment for about eight months with marked improvement, after which artificial pneumothorax of the right side was reinstituted with success, and almost complete collapse was attained except for a portion of the apex held by adhesions. The patient returned to work about 18 months after the first admission into the hospital. Artificial pneumothorax was administered monthly after discharge. About 18 months after discharge for the first illness, the patient had been driving his automobile for a long distance, when he experienced a stabbing pain in the right chest, and upon arrival home was found (by physical examination) to be suffering from spontaneous pneumothorax complicating an artificial pneumothorax with increased intrapleural pressure and a pleural effusion.

The patient was readmitted to the National Jewish Hospital as an emergency case, when physical and X-ray examination revealed spontaneous pneumothorax with hydrothorax up to the fifth interspace on the right side. The left lung showed only slight infiltration of the apex. The manometer gave an intrapleural reading on the right side of 0, +2, and 450 cc. of gas and 100 cc. of slightly turbid fluid were withdrawn. Staphylococcus albus was cultured from the fluid. For two weeks frequent fluid and gas tappings were resorted to. The closed method of pleural drainage was instituted 16 days after second admission. Daily aspiration of from 30 to 150 cc. of pus, and irrigation with Dakin's or salt solution, until the return flow was clear, were found necessary in order to reduce the toxemia. On alternate days 30 cc. of Dakin's solution was left in the pleural space, the patient offering the information that he tasted

t. The daily drainage and flushing were kept up for six months, during which time several intrapleural injections of 100 cc. of a sterile solution of sodium iodide were given for the purpose of locating the bronchial fistula roentgenologically. The X-ray plate taken during the inspiratory phase with the patient lying on the left side showed a band about half an inch wide, with a small projection opposite the sixth rib posteriorly which was inferred to be the bronchial fistula. During this test the patient again volunteered the information that he tasted the sodium iodide, indicating that the fistula was open. The persistance of the bronchial fistula and the progressive loss of weight (48 pounds) in the six months since the second admission made it seem evident that the exacting therapeutic measures so far instituted, though beneficial, were entirely inadequate since the patent fistula was continually reinfecting the pleural space. Since the left lung showed little or no pathological change by physical and roentgenological examinations at this time, and we were influenced by the adverse progress of case 6, thoracoplasty was decided upon as a means of bringing about apposition of the pleural surface to close the pulmonary fistula. As a preliminary measure, phrenicotomy was performed, in order to abolish all involuntary respiratory efforts on the right side and to test the adequacy of the left lung. A few days after phrenicotomy, partial thoracoplasty was performed under novocaine anesthesia, when portions of the eleventh, tenth, ninth and eighth ribs were removed and the drainage-tube was temporarily left *in situ*. The patient gradually improved in strength, appetite and general condition, which made a completion of the thoracoplasty seem inexpedient. He left the hospital five months after operation, weighing 154 pounds although he had reached a minimum of 116 pounds prior to operation. Follow-up records show that at present, 10 months after operation, the patient is in good health and is engaged in his profession.

DISCUSSION

It is surprising that even at the present time one hears that certain groups of tuberculosis therapeutists are opposed to the use of the more radical surgical measures in the treatment of pulmonary tuberculosis, and that even the medical staffs of some of the larger sanatoria are not availing themselves of these methods or making facilities for their use. When the situation is, however, submitted to careful analysis there can be no doubt as to the value of these methods of therapy, but the factor of greatest importance would appear to lie in the careful selection of cases and, once this is determined, to procrastinate is next to professional negligence. There are probably a number of factors which have led to a discrimination against artificial pneumothorax, phrenicotomy and thoracoplasty, the most important of which seems to be the inability

of the individual tuberculosis specialist to choose the suitable case, which is by no means an easy task for the inexperienced; and for the experienced, but unqualified, other factors are blamed when the case goes wrong. Accidents, such as air embolism, cardiac failure, accidental pneumothorax complicating artificial pneumothorax, empyema, etc., are less frequent causes of abstinence, but occur frequently enough to exclude these methods for the less experienced or ultraconservative. An experience similar to that in the series of seven cases cited above leaves one with an everlasting impression of the value of local rest as an adjuvant to the more conservative general rest so long utilized successfully as a therapeutic measure in pulmonary tuberculosis. It is well to follow the plan of beginning with the more conservative measures first, and gradually approach the more drastic when required, as pointed out by Alexander in his recent book (8), but action must not be deferred so long that the patient will have passed the limit when interference will be of no avail. Artificial pneumothorax should not be delayed until marked adhesions have formed, especially in the predominantly unilateral case, nor should thoracoplasty with phrenicotomy be left to be performed when the patient's toxemia has progressed beyond the possibility of even using a local anesthetic, not to mention standing the surgical shock. Thoracoplasty was devised to be used when artificial pneumothorax is indicated but cannot be successfully performed, and as such has its definite place in the therapy of pulmonary tuberculosis. In persistent open or valvular spontaneous pneumothorax complicating pulmonary tuberculosis, thoracoplasty and coincident phrenicotomy have proved especially valuable, and if the mortality from this condition is to be markedly lowered these operations must be performed as soon as possible and before the inevitable pleural contamination has had a chance to gain the upper hand. We are now in an era when timely radical local rest in pulmonary tuberculosis can be justifiably recommended, especially for the cases which are now coming to the sanatorium in greater numbers in the advanced stages and have often been neglected by the man in the field before admission or by the ultraconservative physician in the sanatorium.

The kind guidance of Dr. H. J. Corper in the preparation of this paper is gratefully acknowledged.

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HEALED GENERALIZED MILIARY TUBERCULOSIS[1] [2]

PHILIP H. PIERSON

Technically, the term *miliary tuberculosis* refers to a tuberculous process with lesions of millet-seed size, but through usage it describes a disseminated process throughout the lungs, of lesions of uniform and small size, and spread by way of the blood or lymph-stream. *Generalized miliary tuberculosis* designates the simultaneous distribution of this infection by the blood or lymph to many organs, including the lungs.

The case I am about to report is of special interest because it was generalized, and because its healing proceeded by absorption rather than by calcification. After presenting the data on this case, I shall present briefly the histories and X-ray findings of two others, one of which came under my observation at the Stanford Chest Clinic, and the other was lent to me for its inclusion in this paper by Dr. Eugene Kilgore.

Children and adults tolerate small doses of living tubercle bacilli very well if these are not too virulent or too frequently administered. Such bacilli thereby act as the most powerful immunizer against tuberculosis that we have. But if the dose is too large or too virulent, or our immunity is too low, trouble results, locally if the spread of infection is by contact, and generalized if it is by the blood or lymph. I say "blood" or "lymph" because it is very difficult in some instances to say whether the rupture of the focus is into the blood or into the lymph-stream. From the work of Krause and others, we know that the lymphatic system is the first line of defense in the child. If there is not sufficient resistance to localize the process in a lymph node, bacilli proceed along the lymph-stream, eventually getting into the thoracic duct and thence into the blood-stream. Upon entering the lungs the bacilli do not set up foci in the blood-vessels, but pass through them into the lymph-channels of the lung, whence they may make their way back to the hilum lymph

[1] Read before the San Francisco County Medical Society, San Francisco, California, October 6, 1925.

[2] From the Department of Medicine, Stanford University Medical School, San Francisco, California.

nodes. As we grow older the tuberculous processes tend more and more to be localized in the numerous lymphoid collections found at the bifurcations of bronchi and bronchioles in the lungs. The reason for this is because of the increased fibrosis present there and the increased general immunity which always tends to localize tuberculosis. If immunity is low or lacking, there is no arrest of the process and death generally results.

In this case of mine, a boy of 15, the lymphatic system was so extensively involved that I have raised the question as to whether its spread was by way of the blood-stream or the lymph. We do know that, as lymph-channels become blocked, there is a back-flow of lymph, and, consequently, of the infection it carries.

History: E. C., an Italian boy, age 15, was referred to me by his dentist, because of diseased lymph nodes in his neck. He was seen first in August, 1923. He had been perfectly well until five years before, and since then his only complaint was that he had gained no weight. Tonsillectomy was performed twenty months ago. Eighteen months ago he had had a mild influenza without pneumonia. Following the influenza he noticed the appearance of palpable lymph nodes in each side of his neck, and later in his groins and axillae. These were painless, except for one in the right side of his neck, which gave him a little discomfort. He tired easily, and had some dyspnea on going upstairs, but no edema. There was a slight afternoon fever, but no night-sweats. He was more nervous and irritable than formerly. There was no digestive disturbance, and he ate and slept well. He had no cough, expectoration or chest pain. His past history was unimportant except for three attacks of pain in the right lower quadrant of his abdomen, which possibly were mild attacks of appendicitis or ileocolic adenitis. His family history was negative, including no exposure to tuberculosis.

Physical examination: Showed a well-developed, undernourished boy, whose teeth presented moderate pyorrhoea. His tonsils and adenoids were well removed. The nose showed a deviated septum to the right with poor space. His ear-drums were thickened. *Lymph nodes:* The following nodes showed an enlargement, which was firm, varying in size from half to one inch in diameter, not adherent to the skin but deeply attached, and without redness or fluctuation: anterior and posterior cervical chain, posterior auricular, parotid, submaxillary, supraclavicular, axillary, inguinal and femoral. There were nodules of the same size and type in the skin of the arms and a few on his back, two or three of which were crusted from trauma. His heart was negative except for supracardiac dulness. The lungs showed only interscapular dulness and D'Espine's sign positive to the 8th dorsal vertebra. Blood pres-

sure, 105/70. The abdomen was tense, but masses were felt in the region of the mesenteric and ileocolic lymph nodes. The liver was enlarged to a point two finger-breadths below the costal edge, and the spleen was easily palpable, and both were apparently smooth. Reflexes were normal and there was no edema.

X-ray: Of chest taken August 1, 1923, showed a uniform diffuse mottling throughout the entire lung fields, sparing no part either of the apices or extreme bases. The nodules were 2 mm. in diameter. The hilum shadows were considerably enlarged, showing infiltration of the lymph nodes in both the anterior and posterior mediastinum.

Blood: Hemoglobin, 80 per cent (Dare). Red count, 4,912,000. White count, 5,310. *Differential:* Polymorphonuclears, 64 per cent; large lymphocytes, 13 per cent; small lymphocytes, 15 per cent; transitionals, 6 per cent; basophiles, 1 per cent; and endothelial leucocytes, 1 per cent. Reds slightly pale.

Urine: Three specimens: 1018, acid, heavy trace of albumen, no sugar, 2 to 10 granular and 1 to 3 hyaline casts, and 4 to 10 leucocytes per high dry field; many bacteria. Blood Wassermann negative. Stool negative.

Biopsy of one of the lymph nodes showed destruction of practically the whole of its stroma. This was replaced by miliary tubercles, some showing giant cells and beginning caseation (fig. 7).

I was able to keep the boy in bed for about three weeks, during which time his temperature which was 99.8° at first, gradually reached normal. His pulse varied from 90 to 100, and his respiration was between 20 and 30. After two months of endeavor to keep him under the best hygienic surroundings possible, he disappeared. Not hearing from him for over a year and a half, I supposed that he had died.

During an examination of a large number of high-school boys this spring, I recognized this boy. There was not a lymph node palpable anywhere. The boy had gained 40 pounds in weight and 3 inches in height, and was anxious soon to go out for football, indicative of his own estimation of his health and strength. Chest negative. Liver and spleen not felt.

Description of figures 1 to 6

FIG. 1. E. C. August, 1923.

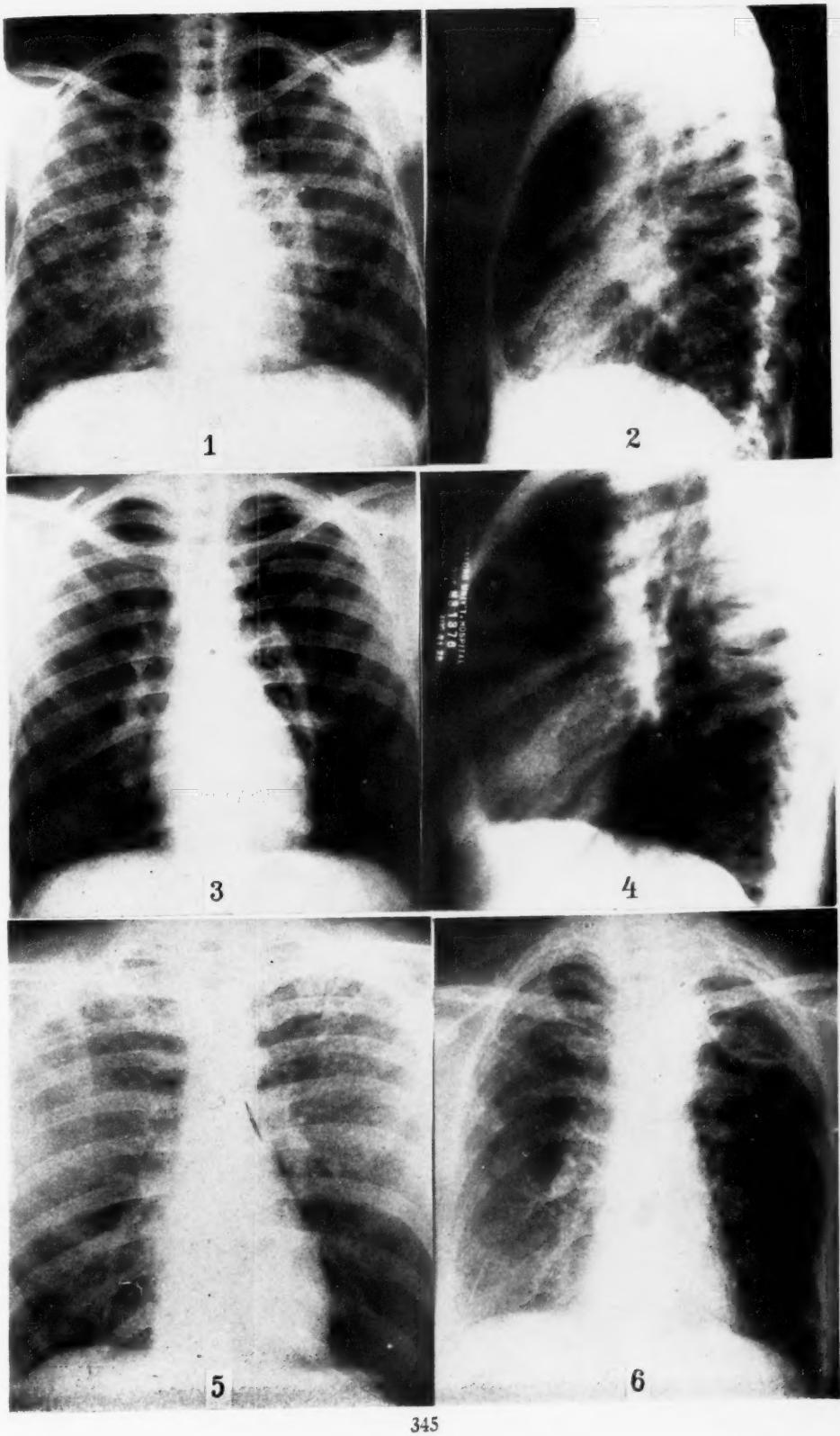
FIG. 2. E. C. Lateral View, August, 1923.

FIG. 3. E. C. August, 1925. Showing absorption of miliary tubercles.

FIG. 4. E. C. Lateral view, August, 1925.

FIG. 5. I. L. X. Miliary tuberculosis, partially sparing lower lobes.

FIG. 6. M. K. Calcified tubercles which because of distribution are considered evidence of healed miliary tuberculosis.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An X-ray taken August 31, 1925, was described as follows by Drs. W. E. Chamberlain and R. N. Newell:

The present films reveal rather extensive hilum scars in the distribution of the bronchial trees. There is nothing to indicate any active lung disease at the present time. Comparing these with the films of 1923, in spite of the remarkable clearing of the process, we are forced to make a diagnosis of widely disseminated tuberculosis of the lungs, and the microscopical findings

FIG. 7. Microscopic section of lymph node of E. C., showing destruction of practically all of the stroma replaced by miliary tubercles, some showing giant cells and beginning caseation.

of tuberculosis of practically all of the lymph nodes, made at that time, justify a diagnosis of miliary tuberculosis, in spite of the outcome. The only other condition which might account for the X-ray appearance of August 1, 1923, is a bronchopneumonia. Such a process would hardly have given the multiple involvement of the lymph nodes, and would probably have healed with less scar tissue.

To summarize this case then, there are four things of special interest:

1. Its involvement of the entire lymphatic system.

2. The complete disappearance of the disease in the lymph nodes, leaving no evidence of its former existence.

3. The clearing of the process in the lungs, leaving only small fibrous scars but no calcification.

4. The possibility of there being very little or no immunity left, in view of so complete a disappearance of the process.

The case lent me by Dr. Eugene Kilgore, is as follows:

Mr. I. L. X.: single, clerk, aged 23. The family history was good. Patient had measles and chicken-pox at 15, mumps five years ago without complications, and "bronchopneumonia" in 1917—sick only a few days. A low-grade fever was present for about two weeks one year ago, and diagnosed as influenza. Six months ago the patient was again sick for a few days with "influenza." Otherwise he has been strong and well, and regular in school. He denied venereal disease. *Habits:* No alcohol, no tobacco, moderate tea and coffee, ordinary habits of eating and exercise. Complained only of cessation, in the last two or three months, of nocturnal emissions, which formerly occurred every week or two. He had not tried sexual intercourse, but was convinced that he had a "weakness." This he blamed to a course of hypodermic injections (staphylococcus vaccine) which a dermatologist gave him about one year ago for acne. He was inclined to be reserved and a bit suspicious toward the medical profession; he thought he was also injured by the typhoid prophylactic injections he received in the army. He may have lost five or ten pounds in the last six months, but he has had no cough, and no disturbance of digestion, bowel-action, urination, sleep, etc.

Examination: Intelligence apparently below the average, although patient had spend some time in university study; he was inclined to introspection, suspicion, etc. Rather slender, not very well nourished, slightly pale. Pupils, mouth, throat, lymph nodes, thyroid, heart, blood pressure, abdomen, reflexes, genitalia, were not remarkable. A slight dulness at the right apex was probably physiological. Otherwise the lungs were clear. The blood showed about a ten per cent reduction in hemoglobin and red cells; otherwise was negative. Urine was clear. Wassermann negative. X-ray showed a fine uniform mottling throughout the upper half or two-thirds of both lungs. A comparison film taken eight months later showed essentially the same condition,—evidently quiescent. Six months later patient's left kidney and epididymis were removed because of tuberculosis; otherwise he remained well.

The third patient consulted us at the Stanford Chest Clinic. She had been north, and on her way back to her home in Arizona, where she

had lived many years, desired to know her present status. Her history was as follows:

M. K.: Born in Ireland, aged 61, married. Patient was apparently in usual good health until eight years ago, when, because of cough, expectoration and positive sputum, she was diagnosed as tuberculous and had taken the "cure" in several sanatoria since then. When seen by us she had been raising a small amount of mucopurulent sputum. (The last sputum positive for tuberculosis was six years ago.) Her weight had remained practically the same for the last five years.

Beside childhood diseases she had pneumonia 39 years ago, and "bronchitis and exhaustion" 13 years ago. Her left breast was removed 10 years ago and no signs of any recurrences were found since (probable diagnosis was carcinoma).

Her family history was negative, except for the death of a son nine months ago from tuberculosis, and a daughter who died after an illness of six months following influenza.

Physical examination was negative, except for her chest, which showed a few fine crepitant râles at her right apex and signs of cavity at her left apex.

Her X-ray showed calcified nodules scattered throughout all five lobes. There was a cavity at her left apex.

In this last case we have the picture of a disseminated tuberculosis throughout the lungs, which healed by calcification except for a cavity at the apex, which may have existed before this disseminated condition took place. In fact, in adult life generalized miliary tuberculosis develops by the breaking through of some focus, frequently a cavity into the blood-stream. Its arrest and healing in this case were due to there being present sufficient immunity to localize the disease. Whether or not any other organs became involved at that time and showed healed tubercles, I cannot say, for it was not possible to make so extensive an examination.

In Dr. Kilgore's case we have another problem. Why were two or three lobes of the lungs involved in a uniform miliary way, while the lower lobes were not so involved? A probable explanation was that there was more blocking along the lymph-channels in the upper lobes than there was in the lower lobes, thereby causing the localization of the process in the minute lymph-spaces at the bifurcation of bronchi and bronchioles. This unequal distribution is the main criticism of considering this a case of miliary tuberculosis.

Other cases of a diffuse tuberculosis in the lungs are seen in which a blood or lymph-borne infection appears quite possible, but these when

localized in one or two lobes are more often due to the bronchopneumonic or contact method of implantation. The most common diseases to consider in differential diagnosis are diffuse bronchopneumonia, pneumonokoniosis, and coccidioidal granuloma. A short period of observation will rule out the first; and with a history of such occupational hazards as produce the second the diagnosis of miliary tuberculosis would be very unwise. Pneumonokoniosis, when associated with tuberculosis, practically always leads to the massive bronchopneumonic type. Coccidioidal granuloma generally breaks down and the microorganisms are found in the sputum.

CONCLUSION

A generalized miliary tuberculosis involving at least the lungs, the lymph nodes and skin, and probably the liver and spleen and possibly the kidneys, has been reported which healed by absorption of the process rather than by calcification.

A number of cases of healed generalized miliary tuberculosis are on record, merely demonstrating that at times there suddenly develops sufficient immunity to overcome a condition generally fatal.

I desire to thank Dr. F. E. Blaisdell for his kindness in making the pathological diagnosis and photograph of section in the case of *E. C.*

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ACUTE TUBERCULOUS LYMPHADENITIS COMPLICATING PULMONARY TUBERCULOSIS

A Report of 3 Cases

ALBERT S. LATHROP[1]

During the past two years we have had three cases of acute tuberculous adenitis in adults as a complication of severe pulmonary disease. Because of the apparent rarity of such cases, we believed that it might be of interest to make a special study of our cases.

Case 1: Female, age 25, housewife. Admitted to Sanatorium November 6, 1922. *Complaint:* Loss of strength, fever, night-sweats. *F. H.:* Unimportant in relation to tuberculosis. *P. H.:* Healthy childhood under good circumstances. Had measles, mumps, whooping-cough, chickenpox and malaria in childhood. Married four years ago. One child, 17 months old. No other pregnancies until present time. *P. H.:* Patient had two severe chest colds at 15 years of age, each lasting three to four months, characterized by severe cough, considerable expectoration and marked loss of weight. She has felt well since, until three years ago when she felt run down. Chest examination at that time was negative, but X-ray showed definite pulmonary tuberculosis. She rested four months, rapidly recovering her strength. She had a normal pregnancy 17 months ago. During the puerperium she felt fatigued and began to lose weight, nursing her baby, however, for 7 months. Her strength has been below par since. During the summer she had marked digestive disturbances, characterized by gas and distention. For the past three weeks she has had a temperature to 103° at night, with constant dyspnea and one drenching night-sweat. She has had no pain in her chest, and no hemoptysis, cough or expectoration. She was five months pregnant on admission. On admission her temperature ranged to 100° and 101° in the afternoon, pulse 100 to 120; normal weight 112, standard 121, previous high 120, admission 111. No sputum for examination; urinalysis negative.

P. E.: Well-nourished woman of 25; enlarged lymph nodes in left anterior cervical chains and left axilla. Slight systolic murmur at pulmonic area. *Lungs: Right:* Note impaired above clavicle and third vertebral spine. Bronchovesicular breathing above clavicle and second vertebral spine. Increased whisper above clavicle, in second interspace and above scapula. No

[1] Gaylord Farm Sanatorium, Wallingford, Connecticut.

râles. *Left:* No impairment of note, no change in breath-sounds; increased whisper above clavicle and second vertebral spine. Slight friction rub at base. *X-ray: Right:* Active infiltration from apex to third rib and third vertebral spine, intense in apex. *Left:* Slight, acutely active, scattered infiltration from apex to second interspace and at fourth and fifth vertebral spines.

Diagnosis: Moderately advanced, C-I; R-u; L-u, l.

The patient was kept in bed at strict rest for three months. Her temperature continued to around 100° each afternoon; tubercle bacilli were found in her sputum on one examination. In December, 1922, she had an acute painful swelling of the lymph nodes in the left axilla. Examination showed a firm, irregular, painful mass of lymph nodes without any tendency to soften or break down. These gradually decreased in size.

The patient was transferred to the hospital February 10, 1923. A healthy baby was born March 27th after an easy labor. She ran a temperature to 102°, though feeling well, for ten days after delivery, when she had a sudden attack of abdominal distention ushered in by a slight chill, and associated with general enlargement of superficial lymph nodes, most marked in both inguinal and axillary regions, and with swelling of both legs below the knees. She had no rise in temperature above the previous level, until the middle of May when she had an attack of dry pleurisy on the left side, with a temperature to 104° for three days and severe pain in the mass of lymph nodes in the left axilla. Otherwise her condition remained about the same until she was readmitted to the Sanatorium on July 18, 1923, with temperature at 100° each afternoon. At this time her chief complaints were slight cough and expectoration, marked weakness, and enlargement of the superficial lymph nodes. Her physical examination at this time recorded general enlargement of superficial lymph nodes, the largest mass being in left axilla, and moderate edema of both ankles. *Lungs: Right:* Note impaired above second rib and fourth vertebral spine with scattered medium moist râles in this area. *Left:* Note impaired above second rib and third vertebral spine and below eighth vertebral spine. Scattered medium moist râles between second and fourth vertebral spines. *X-ray:* Increase in area, intensity and activity of lesion on both sides, now showing to third interspace on right and fourth rib on left. Urine examination negative; sputum negative.

The patient was kept at strict bed-rest for three months, with gradual disappearance of her cough, return of strength, and gain of 15 pounds. The enlargement of the lymph nodes persisted, though pain disappeared. The temperature gradually subsided to normal; it was below 99° for the last month of bed-rest. Physical examination at this time was the same as on readmission except that the edema of ankles had disappeared.

A small superficial lymph node in the right supraclavicular fossa was

removed for examination. This had a typically tuberculous appearance; caseated with a small rim of lymphoid tissue containing scattered giant cells. The patient had been taking artificial heliotherapy since readmission and now was placed on small doses of tuberculin *B. E.* weekly, starting with 0.000,000,025 gm. From this time on there was a gradual decrease of moisture in her lungs and a rapid decrease in the size of the lymph nodes. The patient was given gradually increasing exercise, starting with meals on November 11, 1923, and on 15 minutes' exercise December 17. Her improvement continued; she was discharged from the Sanatorium April 12, 1924, at which time she was taking one hour's walk twice a day without fatigue and had no chest symptoms.

Physical examination at that time showed a marked decrease in size of all superficial lymph nodes, most marked in the cervical region, and entirely disappearing on the right side. The nodes in her left axilla were now small, discrete and freely movable. *Lungs: Right:* Note impaired above clavicle and third vertebral spine; no râles. *Left:* Note impaired above clavicle and fourth vertebral spine; breathing faint above clavicle, and irregular and harsh in the first interspace and above the third vertebral spine; no râles. The X-ray showed marked improvement, though the case was still considered slightly active.

The patient did well after discharge, leading a normal life except for two hours' rest each afternoon until February, 1925, when she had pneumonia and empyema on the left side. With this she again had a general lymphadenitis, most marked in both inguinal regions, and with moderate swelling of both ankles. This has gradually disappeared. After a slow return of strength, she is now at home, apparently as well as before her attack of pneumonia and empyema.

Case 2: Male, age 25, occupation office clerk, single, admitted January 3, 1924. *Complaint:* Cough and expectoration, pain in right lung, gas in stomach. *F. H.:* One brother, age 20, died one year ago, probably of tuberculous meningitis. No other history suggestive of tuberculosis. *P. H.:* The patient had a healthy childhood up to 7 years of age when he had pneumonia and empyema on the left side. A second attack of pneumonia occurred at 9 years (side not known) and a third attack at 16 on the right side. After finishing high-school at 17 the patient started office work and has done that since. He has always been susceptible to head and chest colds. He has had dyspnea on slight exertion since childhood. *P. I.:* In the fall of 1920 he did not feel well and had several drenching night-sweats. After several months he again felt well until August, 1923, when he began to feel increasingly tired. In November he had a severe cold, with dry cough and severe headaches, but without expectoration. The cough gradually grew worse, becoming paroxysmal, and the patient began to feel feverish. He had

repeated night-sweats early in December, and blood-streaked sputum once. He was admitted to a general hospital December 12, 1923, where a diagnosis of pulmonary tuberculosis was made; he was then transferred to Gaylord Farm.

On admission to the Sanatorium he had severe cough, profuse mucopurulent expectoration, marked fatigue, loss of weight, night-sweats, fever, hoarseness and considerable discomfort from flatulence. His temperature after admission ranged from 100° to 102°; pulse 100 to 120; normal weight 135, previous high 145, standard 154, admission 136; sputum positive, Gaffky V; urinalysis negative. *P. E.:* On admission: Patient appears ill; a few enlarged lymph nodes in right anterior cervical chain. *Lungs: Right:* Note impaired above clavicle and fourth vertebral spine; breathing faint over same area; a few scattered râles after cough above second rib. *Left:* Note impaired to clavicle and fourth vertebral spine; increased whisper to clavicle; numerous medium moist râles above second rib; a few rhonchi over upper back. *X-ray: Right:* Pleurisy over side; infiltration to third rib and outer third interspace; activity slight. *Left:* Infiltration from apex to fourth rib; active.

Diagnosis: Moderately advanced C-I; R-u; L-u.

The patient was kept under strict bed-care for three months, during which his temperature ran to 103.2° at the peak of wavy variations; 98.8° was the lowest afternoon temperature. The pulmonary tuberculosis slowly advanced during this time, with persistent cough and expectoration; the digestive disturbance cleared up, and the patient gained 15 pounds in weight. Three weeks after admission a large boggy swelling appeared in the right side of neck, but with very little soreness or tenderness. The leucocyte count was 8,300, with polymorphonuclears 68 per cent and lymphocytes 32 per cent. An ice-bag was applied to the neck, and after three or four days the swelling partially subsided. At this time two large lymph nodes were distinctly palpable, with two smaller ones below them. The swelling persisted about the same from then on until discharge. Because of the fever no heliotherapy or tuberculin was given. The patient was discharged April 18, 1924, with a poor prognosis and with directions to continue under strict bed-rest at home. He remained in bed at home for 6 months, with loss of fever, decreased cough and expectoration, and diminution of the mass in right cervical region. He was given gradually increasing exercise for two months, when he had a relapse, and has been running a fever since, with increasing physical signs in the chest. The largest lymph node in the right cervical region broke down at this time and has been draining since.

Case 3: Male, age 27; single, occupation physician, admitted April 12, 1924. *Complaint:* Cough, expectoration, night-sweats and loss of weight. *F. H.:* Negative. *P. H.:* The patient was rather frail as a child. He had measles,

mumps, whooping-cough and chickenpox during childhood; a mild attack of influenza in 1919, and scarlet fever in 1921. A herniotomy and appendicectomy were done under ether in 1922. He graduated from medical school in 1921, and has done hospital work since. *P. I.:* In December, 1923, he felt tired, especially in the afternoon. In January, 1924, he began to have a dry paroxysmal cough, with expectoration commencing a month later; tubercle bacilli in the sputum were found during the last week of March. The patient had no hemoptysis or blood-streaked sputum. During the five weeks previous to admission he has felt flushed each afternoon, has had several night-sweats, and has lost 6 pounds. He has had occasional sharp pain in lower right axilla on cough. His temperature went to 101° two weeks ago; no temperature record has been kept.

On admission the patient's temperature ranged between 100° and 102°; pulse 80 to 100; high weight 174, normal 150, standard 167, on admission 143; sputum positive, Gaffky VI. *P. E.:* Fairly well-nourished, pale, acutely ill man; teeth in fair condition, several crowns; tonsils small, ragged; a few small palpable lymph nodes in left cervical region; heart normal. *Lungs: Right:* Note impaired above fourth rib and over back; bronchovesicular breathing above second rib and fourth vertebral spine; marked increase in whispered voice above third vertebral spine; amphoric breathing and whisper above scapula; numerous moderately coarse râles on ordinary breathing above fourth rib and sixth vertebral spine, after cough over front and above sixth vertebral spine. *Left:* Slight impairment of note above clavicle and third vertebral spine; a few fine dry râles after cough above third vertebral spine. *X-ray: Right:* Dense active infiltration from apex to fourth rib and eighth vertebral spine. *Left:* Small amount of active infiltration deep in and under the fourth interspace; a few discrete tubercles in apex.

Diagnosis: Pulmonary tuberculosis; far advanced, C-II; R-u, m, l; L-u.

Two weeks after admission an acute swelling appeared on the left side of neck, forming an irregular, hard, tender mass without increased signs of general toxemia. An ice-bag was applied at intervals for several days. During this time the tenderness subsided, but there was very little change in the size of the mass. Because of a rapidly spreading lesion in the right lung, artificial pneumothorax was begun May 11, 1924, and has been continued to date. The patient's general condition remained unchanged for a few months; then the temperature gradually subsided and he had an increased appetite with a gain in weight, associated with gradual decrease in the size of the mass of lymph nodes in the left cervical region. In September the patient was started on heliotherapy. In October strict bed restrictions were removed and he has been gradually allowed up; he is now on 30 minutes' exercise twice daily. He has had no cough or expectoration during the last two months, and has gained 25 pounds since admission. Since starting heliotherapy

the subsidence of the mass in his neck has been continuous and fairly rapid. He now wears a collar of the same size as at the time of his best previous weight, with only a slightly visible difference in the two sides of neck. Palpation shows the lymph nodes to be discrete, freely movable, and only slightly enlarged, except for one in the lower part of the cervical chain, which is still the size of a walnut, hard and freely movable.

In these three cases acute lymphadenitis developed in patients with an acutely spreading pulmonary lesion. Both cases of cervical involvement had previously had enlarged lymph nodes in this region, though without any history of adenitis.

The case of generalized lymphadenitis presumably had a bacteremia, with tubercle bacilli entering the blood-stream from a focus in the pulmonary circulation, and then filtered out from the capillaries into the lymph nodes. The node in the right supraclavicular fossa that was excised for study had not been palpable before the acute onset; so presumably it was in the same condition as the other acutely enlarged nodes. The intestinal upset that was coincident with the enlargement of the lymph nodes was similar to several previous attacks and consequently was probably due to the toxemia, although it might have been caused by an acute tuberculous infection of the mesenteric lymph nodes. The swelling of the patient's legs and feet was also coincident with the enlargement of the lymph nodes. She had no physical signs of phlebitis or any rise of temperature above the level she had been maintaining. This edema could be explained by obstruction of the return lymph-flow in the enlarged nodes or by their pressure obstructing the venous flow.

The differential diagnosis in the two cervical cases lay between mumps and acute cervical adenitis. In these two cases the swelling was too low, being entirely below the angle of the jaw, nor was there any change in the salivary flow. The course of the illness also ruled out mumps. In the case of the general enlargement of the lymph nodes, Hodgkin's disease and leukemia were considered. The spleen was not enlarged, nor was there any change in the blood picture. Hodgkin's disease was not ruled out until the section of the excised lymph node showed a typical picture of tuberculosis.

Case 1 was started on artificial heliotherapy on readmission and continued on it until discharge. She was also given tuberculin *B.E.* as soon as a definite diagnosis of tuberculous lymphadenitis was made,

and with it showed rapid improvement in her pulmonary as well as lymph-node tuberculosis.

Case 2, because of the high fever, was given neither heliotherapy nor tuberculin.

Case 3 has been taking heliotherapy on the sun-porch when the weather permits. During most of the winter he was on artificial heliotherapy. Because of the rapid improvement in his condition he was not given tuberculin.

At onset we considered that all three had a poor prognosis, based on their pulmonary tuberculosis alone. Whether or not the lymphadenitis affected the course of the illness is difficult to say. Soon after the onset of the acute lymphadenitis in two of the cases, the progression of the pulmonary disease ceased and slow improvement began. This could have been expected from the course of intensive bed-rest all three were given. In none of the cases did the lymphadenitis impair the prognosis.

SUMMARY

Three cases of acute tuberculous lymphadenitis complicating pulmonary tuberculosis are presented. In two of the cases the adenitis involved only the cervical nodes on one side; in the third case all palpable lymph nodes were involved. In none of the cases was the prognosis definitely altered by the presence of the complication.

THE CUTANEOUS (MORO) TUBERCULIN TEST IN ARTIFICIAL-PNEUMOTHORAX PATIENTS[1]

HERBERT F. GAMMONS

A few years ago, while making routine tuberculin tests, I was impressed by the fact that those who gave a poor prognosis showed a persistently negative reaction.

It occurred to me that it would be of interest to use the test on a number of patients before administering artificial pneumothorax, and, at intervals during the treatment, to ascertain what change, if any, might occur in the tuberculin reaction as a result of compressing the lung.

With the idea in mind that a negative tuberculin reaction before compression might become positive after the lung was collapsed, due to a decrease of intoxication, I tested all cases that I considered needed pneumothorax treatment, and tests were made before and during the treatment, after a good compression was obtained.

The cutaneous (Moro) tuberculin test was used, as it was thought that this method offered the best opportunity of studying the reaction without hurting the patient. Nine patients were given the test before treatment. A brief description of these follows:

Case 1: C. A. *Right lung:* Post-tussive coarse râles throughout, with amphoric breathing at top. *Left:* Post-tussive medium coarse râles above second rib and third dorsal spine. Expectoration large in amount and with many tubercle bacilli. Temperature averaged 101°F. in the evening daily. Very much underweight. X-ray showed the entire right lung active, and activity above the second rib on the left. Skin test was negative. Artificial pneumothorax administered with an improvement at first, but patient developed intestinal tuberculosis and gradually became worse. His right lung was well compressed.

Case 2: H. V. Left lung completely involved, with scattered infection in upper lobe of right. Hemorrhages were frequent, apparently from the left lung. Temperature from 100° to 102° daily. Cough very troublesome, with sputum in large amount, containing many tubercle bacilli. Tuberculin test

[1] From the Arkansas State Tuberculosis Sanatorium, Booneville, Arkansas, Dr. John Stewart, Superintendent.

negative. On account of hemorrhages an X-ray examination was not made. After three treatments the patient developed a left-sided spontaneous pneumothorax, which was complete and valvular. After many aspirations of air the patient died.

Case 3: J. B. *Right lung:* Slight infection in the upper right lobe. *Left lung* was completely involved, with cavitation at apex. Temperature ranged between 101° and 103°. X-ray examination showed slight mottling in the right upper lobe, with extensive mottling throughout left. Cough severe, and sputum excessive and contained many bacilli. Skin test negative. Pneumothorax treatment started with an improvement of all symptoms at first. After the lung was well compressed trouble developed in the opposite lung, and the patient rapidly became worse and died.

Case 4: J. T. Left lung completely filled with medium coarse râles. Right lung appeared negative. Larynx was infected, the true and false vocal cords being inflamed and ulcerated in places. X-ray showed mottling throughout left, with some fibrosis in the right upper. Skin test negative. Temperature continually high. Cough severe. Pneumothorax treatment started with marked improvement of symptoms. After the lung was very well compressed, the patient developed a severe bronchitis and, as he showed so much more trouble in his good lung and his laryngeal infection was worse, it was thought best to stop the pneumothorax treatments.

The above cases all had a negative skin test at the beginning of treatment and at intervals during treatment, and in three when the lung was compressed thoroughly.

The following cases showed a positive skin test before starting pneumothorax treatment, and the test was more positive after the lung was compressed:

Case 5: H. B. Right lung active above fourth rib and sixth dorsal spine. The lung in this region was almost consolidated. Left lung, slight infection above second rib. X-ray examination showed the same extent of trouble as discovered clinically, with a deep cavity in the right upper lobe. Sputum positive and increasing in amount. Temperature averaged 100°F. daily. This patient had been taking sanatorium treatment for six months, and was getting worse. Skin test positive. At present, after six months' treatment, the lung is well compressed and the patient has no signs of activity.

Case 6: M. A. Patient had been taking sanatorium treatment for nine months, but after taking a little more exercise than he had been accustomed to he developed cough and an increase in expectoration, and began having

hemorrhages, apparently from the right lung. The right lung was full of medium coarse post-tussive râles, with a cavity at the apex, and the left showed a little activity above the third rib. X-ray examination confirmed the physical findings. Skin test positive. The patient has practically no symptoms of activity and has gained from 86 to 98 pounds after four months' treatment.

Case 7: N. B. This patient had an infection of the entire right lung, with cavity at the apex. The left lung showed slight activity above the second rib. After a short period of observation the patient was given pneumothorax treatments, as her cough was almost incessant. Expectoration increasing and fever persistent. The skin test was positive. This patient has a good compression and is free from all symptoms.

Case 8: H. P. This patient had been taking the treatment for over a year, and had had hemorrhages at frequent intervals during this time. The left lung was completely infected and the right was active above the third rib. Temperature varied, but cough and expectoration were very bothersome. Skin test positive. Pneumothorax was administered on the left side, and patient is now in excellent condition and has gained twenty-five pounds in weight.

Case 9: G. R. This patient had an infection in the entire right lung, and a scattered infection in the left upper two-thirds. The disease was active in both lungs, but the right was so much worse that it was deemed advisable to compress this lung as the patient was not improving. The temperature was increasing. Also cough and expectoration. Skin test was positive. Pneumothorax treatment started, and the patient has made a very good improvement in all respects.

Previous to starting the tuberculin tests on patients taking artificial pneumothorax, I had treated 210 with this method of treatment, but unfortunately I did not test all of them with tuberculin.

It is realized that the small number of cases here reported is not sufficient to base any conclusions upon, but it seems that further work by all operators would give us, perhaps, conclusive evidence regarding the value of the skin test in the selection of pneumothorax patients.

Those patients with negative reaction before artificial pneumothorax was begun showed a negative reaction during and after the treatment. Those patients in whom the skin test was positive before treatment showed a more marked reaction after the lung was compressed. The negative skin-test cases have died or are expected to die, while the positive skin-test cases give all hopes of getting practically well.

THE RETICULUM OF THE LUNG

III. ITS RÔLE IN THE HEALING OF MILIARY TUBERCLES

WILLIAM SNOW MILLER[1,2]

In a previous contribution the relation of reticulum to early tuberculous processes in the human lung was described. It was there shown that associated with the development of a miliary tubercle there is an increase in the amount of reticulum, and that reticulum was to be considered a precollagenous type of tissue which by further development was converted into collagenous (fibrous) tissue.

This was followed by a preliminary report, in which the relation of reticulum to the later stages of pulmonary tuberculosis was briefly discussed, and it was there stated "that in the formation of reticulum and its conversion into collagenous tissue a healing process is taking place." The present contribution will take up the latter thesis, leaving the main topic of the preliminary report for a future communication.

In figure 1, a microscopic tubercle is seen which contains in its centre a giant cell that is surrounded by a network of fine reticulum; around the periphery of the tubercle there are coarser fibres which, in a few places, show a tendency to group themselves into bundles. These latter fibres do not, in the stained section, show the characteristic color of fully developed collagenous fibres; they are intermediate in color between reticulum fibres and collagenous fibres. They must, therefore, be considered as collagenous fibres in a transitional stage of development.

While in figure 1 caseation has not taken place, in figure 2 the central portion of the tubercle has become caseous. This caseous area is surrounded by, and permeated with, a network of fine fibrils of reticulum. The network within the caseation can be seen in the two light areas within its central portion. The fibrils of reticulum, especially about the area of caseation, have become thickened and, like those in figure 1, are gradually being converted into collagenous fibres.

In figure 3 the caseation, which at one time was present in the central

[1] University of Wisconsin, Madison, Wisconsin.

[2] Aided by a grant from the National Tuberculosis Association.

portion of the tubercle, has nearly disappeared. The reticulum which permeated the caseation has not been broken down, but has advanced in its transformation into collagenous tissue. The dense network of fibrils which surrounds the tubercle is composed of both reticulum and fully developed collagenous fibres.

It occasionally happens that there comes into the hands of the investigator a lung which shows throughout its entire extent the various steps by which the reticulum of individual tubercles is converted into collagenous tissue. A longitudinal section through such a lung is shown in figure 5. A brief abstract of the history which accompanied the specimen follows:

Male, white, age 56 years. Was under observation between four and five years. During that time he had a persistent cough with but little expectoration. No history of hemorrhage. No bacilli were demonstrated at any time while he was under observation. Any exertion occasioned shortness of breath. No night-sweats. No marked loss of weight. X-ray examinations were made at various intervals with practically the same findings in each instance; namely, marked mottling of pronounced density throughout both lungs. The X-ray plates taken shortly before he died showed no marked difference from those taken four years previously. Clinically, he presented at the end the picture of a patient with cardiac failure, edema of lower extremities, and ascites. The necropsy showed that in addition to the pulmonary tuberculosis there was an hypertrophy of the right ventricle and a general passive congestion of all the viscera.

A photomicrograph of a section from the lung illustrated in figure 5 is shown in figure 4. A study of the six tubercles which appear in the section reveals the time-relation of their development. The section was stained with Bielschowsky's silver. This stains all fully developed collagenous fibres a golden brown, reticulum a pronounced black, and transitional fibres, that is, fibres in which the collagen is not fully formed, a gray or brownish color, depending on the stage of development. By using a proper color-filter, when the photographic plate is exposed, the collagenous fibres appear in the finished print a light gray, while the reticulum remains black; transitional fibres also appear black in the print.

The youngest of the tubercles, *1*, figure 4, is readily recognized by its nearly uniform black color. Around its upper right margin there are a few collagenous fibres which have imparted a lighter tint to this por-

tion of the tubercle. This tubercle represents the next stage after the one shown in figure 3.

The next tubercle in age is the one which was divided when the block was cut out, *2*, figure 4. The centre of this tubercle is of a uniform black, while the periphery shows a mixture of collagenous fibres and reticulum.

The tubercle at *3*, figure 4, is slightly older than the one at *2*; this is shown by the lighter color of its periphery, and the extension of the collagenous fibres into its central portion, giving a lighter tone to this part of the tubercle.

At *4*, figure 4, the collagenous fibres around the periphery of the tubercle have increased in number, and its central portion is largely made up of collagenous fibres, though there are still many fibres of reticulum present.

There is, in the central portion of the tubercle at *5*, figure 4, a uniform mixture of collagenous fibres and reticulum which gives to it a

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Description of figures

If the photomicrographs be studied with the aid of a strong reading glass, many of the finer details can be seen more clearly. Figs. 1 to 4 are stained with Bielschowsky's silver stain. Fig. 5 is unstained.

FIG. 1. A microscopic tubercle showing a network of fine fibres of reticulum in its centre; coarser fibres, which have not been wholly converted into collagenous fibres, surround its periphery. × 160.

FIG. 2. A tubercle in which the central portion has become caseous. Fibres of reticulum are present throughout the caseation. Surrounding the caseation there is an area made up of coarse fibres of reticulum, while around the whole there is a network of collagenous fibres. A single, shrunken giant cell is present in the upper left quadrant. × 60.

FIG. 3. The caseation which formerly occupied the centre of this tubercle has practically disappeared and has been replaced by reticulum. The heavy, dark, fibres which surround the central portion are collagenous fibres that were stained a yellow-brown by the silver. No screen was used in taking this photograph; therefore, the collagenous fibres appear black. Between the heavy black fibres, and often connected with them, are fibres of reticulum. × 80.

FIG. 4. A section from the lung shown in fig. 5. The numbers *1* to *6* designate tubercles in which various stages in the conversion of reticulum into collagenous tissue can be recognized. This section has been described in detail in the text. × 4.

FIG. 5. Longitudinal section through the right lung of a case in which miliary tubercles have been arrested in their development. The characteristics of the tubercles are shown in fig. 4. The *incisurae* between the lobes of the lung can be recognized; the one between the middle and lower lobe is especially clear, on account of the adhesions. There is a cut through the upper lobe, and the place where one of the several blocks of tissue had been taken for sectioning shows in the photograph. Photograph reduced to four-sevenths of the original size of the lung. Lantern slides from this lung were shown at the twenty-first meeting of the National Tuberculosis Association, 1925.

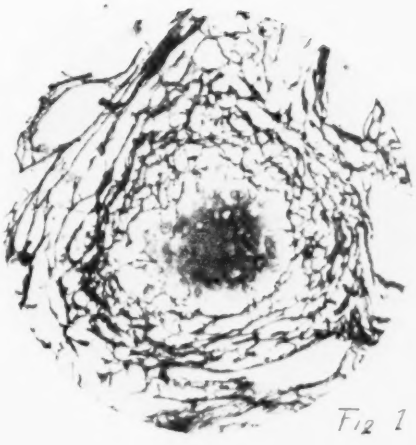

Fig. 1

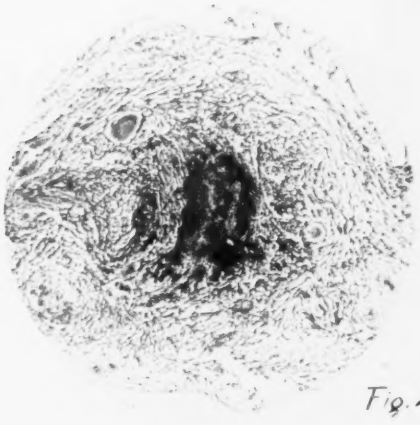

Fig. 2

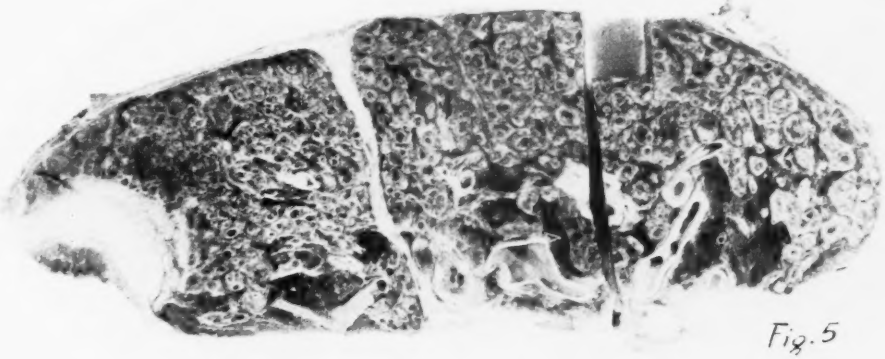

Fig. 5

Fig. 3

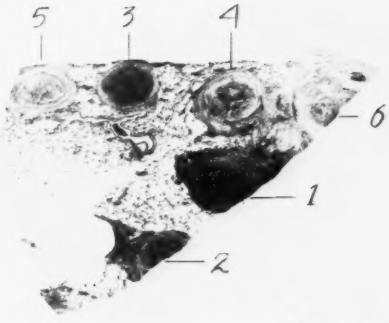

Fig. 4

fine, stippled appearance, while the periphery is wholly made up of collagenous fibres.

In the tubercle at *6*, figure 4, practically the entire tubercle has been converted into collagenous tissue; scarcely a single fibre of reticulum can be distinguished under the microscope.

Between *4* and *6*, and between *1* and *6*, figure 4, portions of two other tubercles can be seen which are undergoing changes similar to those described above, while in other situations an emphysema is present.

DISCUSSION

It is questionable whether a tubercle like the one illustrated in figure 1 would become caseous. If there should be an increased growth of bacilli with an influx of polymorphonuclear leucocytes, caseation would probably occur; if, however, the mononuclear leucocytes succeed in overcoming the infection, an increased development of reticulum will take place, and its conversion into collagenous tissue, a procedure that has already begun around the periphery of the tubercle in figure 1, will follow.

In figure 2 caseation had taken place. The destructive process inaugurated by the continued growth of bacilli and the accumulation of polymorphonuclear leucocytes has been arrested, and mononuclear leucocytes have wandered into the centre of the tubercle from its periphery, giving rise to a further increase in the amount of reticulum. The caseation is gradually being removed, and collagenous fibres are developing around the periphery of the tubercle.

With the disappearance of caseation, the mononuclear leucocytes are increased in number. These factors lead to a still further development of reticulum, and, with a cessation of the destructive process associated with caseation, to its being converted into collagenous tissue. The tubercle is now fairly started in a healing process, as in figure 3.

It is very evident that the tuberculosis represented in figure 5 had existed many years longer than is indicated in the history which accompanied the specimen. All the lobes of the lung are involved, but there is a small area just above the diaphragmatic surface of the lower lobe in which the tubercles appear to be of a more recent growth than elsewhere. The nontuberculous areas vary in size, the largest being in the upper lobe. Blocks of tissue were removed from various portions of the lung for sectioning; only one of the places from which they were

taken appears in the photograph. All the sections presented the same general characteristics.

The microscopic section shown in figure 4 needs no further discussion than that which has already been given. The tubercles and their order of growth have been sufficiently described; they complete the story of the rôle which reticulum plays in the healing of miliary tubercles.

CONCLUSIONS

1. Reticulum is a precollagenous type of connective tissue.
2. In the conversion of reticulum into collagenous tissue a healing process is taking place.
3. Whatever tends to promote the formation of reticulum and its conversion into collagenous tissue tends to promote the healing of a tubercle.

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THE INFLUENCE OF WINTER CLIMATE ON PULMONARY TUBERCULOSIS IN THE UNITED STATES[1]

JOHN RICE MINER

The view has been expressed that the poor ventilation of houses consequent on a severe winter climate is an important factor in the causation of pulmonary tuberculosis. Thus Greenwood (1) in this recent paper, *The Vital Statistics of Sweden and England and Wales*, writes as follows (p. 509):

> A small but not unimportant matter is that the overcrowding of living-rooms . . . . must, from the point of view of such infections as tuberculosis (which we shall soon see is a graver evil in Sweden than in his country [England]), be more deleterious than in England. The Swedish climate is more severe than that of England and Wales, and to cope with the severity of the winter most houses have double windows. To this circumstance, the close aggregation of human beings in rooms sealed against the cold outside air, the heavy incidence of phthisis upon, e.g., Esquimaux, has been attributed.

In the discussion on the same paper Baines also said (*loc. cit.*, p. 533):

> In regard to the connection between housing and phthisis, the climate possibly plays a leading part. The winter is long, the cold intense outside, and the crowded living-room, kept very hot and being used all day, is never efficiently ventilated, as the cottage rooms are here. The great prevalence of this disease amongst the Eskimo has been attributed to this cause, I believe.

The weakness in the examples adduced to support this view is the difference in racial composition of the populations compared, as well as in environmental factors other than climate. In these respects the mortality statistics for the white population of the United States Registration Area offer perhaps unique advantages, covering as they do a climatic range from the rigors of a Minnesota or Montana winter to the semitropical climate of Florida, yet referring to a population fairly homogeneous in racial composition and highly homogeneous in customs and social environment.

[1] From the Institute for Biological Research of the Johns Hopkins University, Baltimore, Maryland.

TABLE 1

Rural white death-rates per 100,000 for 1920 from tuberculosis of the lungs in United States registration states, grouped by January mean temperature

January mean temperature					
UNDER 20°F.	20° TO 25°F.	25° TO 30°F.	30° TO 35°F.	35° TO 40°F.	OVER 40°F.
Maine.....82.5	Massachusetts. 124.0	Rhode Island..173.0	Delaware.....136.2	Virginia..... 82.2	North Carolina...64.9
New Hampshire..90.4	New York.....114.9	Connecticut.... 84.2	Maryland.....104.8	Tennessee.....114.9	South Carolina...55.0
Vermont.....53.3	Michigan..... 62.4	New Jersey....115.2	Kentucky.... 114.1	Oregon..... 60.5	Florida.....54.7
Wisconsin.....71.5		Pennsylvania.. 80.9	Missouri..... 88.7		Mississippi..... 41.1
Minnesota.....66.7		Ohio..... 79.1	Washington.... 80.1		Louisiana.....48.3
Nebraska.....25.8		Indiana..... 90.2			
Montana.....43.7		Illinois..... 88.7			
		Kansas..... 29.6			
		Utah..... 15.9			
Weighted mean death rate					
61.1 ± 0.7	96.7 ± 1.0 62.4 ± 1.3‡	80.4 ± 0.5 77.0 ± 0.5§	99.0 ± 0.9	94.7 ± 1.1	54.9 ± 0.8* 49.1 ± 0.9†

* Including North Carolina.

† Not including North Carolina.

‡ Not including Massachusetts and New York.

§ Not including Rhode Island and New Jersey.

Table 1 gives the rural white death-rates for 1920 from tuberculosis of the lungs and acute miliary tuberculosis in the various registration states grouped according to January mean temperature, as well as the weighted mean death-rate for each group. The distribution of the states was based on plate XI of A. J. Henry's *Climatology of the United States* (2), showing the January isotherms. Where a state was crossed by a limiting isotherm and might therefore be assigned to either of two classes, it was placed in the class representing the conditions to which, on the average, the rural population was exposed. The comparison is restricted to rural populations, not only because they are racially more homogeneous than the urban, but because their living conditions are more dependent on climatic factors. In spite of the vigorous preaching in late years of the gospel of the open window, anyone acquainted with the rural portions of northern New England knows that double windows and storm-doors are as much part of the customary winter equipment of a household as overcoats and overshoes, and that as little outside air as possible is allowed to enter the house during the winter. In general, there can be no doubt that the inhabitants of regions with rigorous winters live on the whole in poorer conditions of ventilation than those enjoying milder climates. California and Colorado are not included in the table, since the large number of consumptives who go to these states seeking health would vitiate any comparison. Although this objection does not, I believe, apply to the same degree to rural North Carolina, the mean death-rate for the group of states with temperatures over 40°F. is given both with and without North Carolina.

In accordance with the practice of the Census Bureau in tabulating mortality statistics the term *rural* indicates the area outside of municipalities having a population of 10,000 or more in 1920. The populations used in calculating the death-rates are therefore the sums of the "rural" populations as given in the Census and of the cities of 2,500 to 10,000 inhabitants. The rates are not adjusted for age and sex, since the necessary data are not available, but as comparisons are made only between rural areas, among which age and sex distribution does not vary greatly, this is not a serious disadvantage.

As will be seen from the above definition of "rural," in highly industrialized states, such as Massachusetts, Rhode Island, New York, and New Jersey, a not insignificant portion of the technically rural population consists of the inhabitants of mill or factory towns, and of the regions around large cities but not included within the city limits. The occupied

portions of these populations do not lead a rural life. They are exposed to many industrial hazards favorable to tuberculosis and are not properly comparable with the strictly rural dwellers. The weighted mean death-rates for temperature classes 20°–25°F. and 25°–30°F. are therefore given, both including and excluding these states.

The mean death-rates for the six temperature groups of states are shown graphically in figure 1.

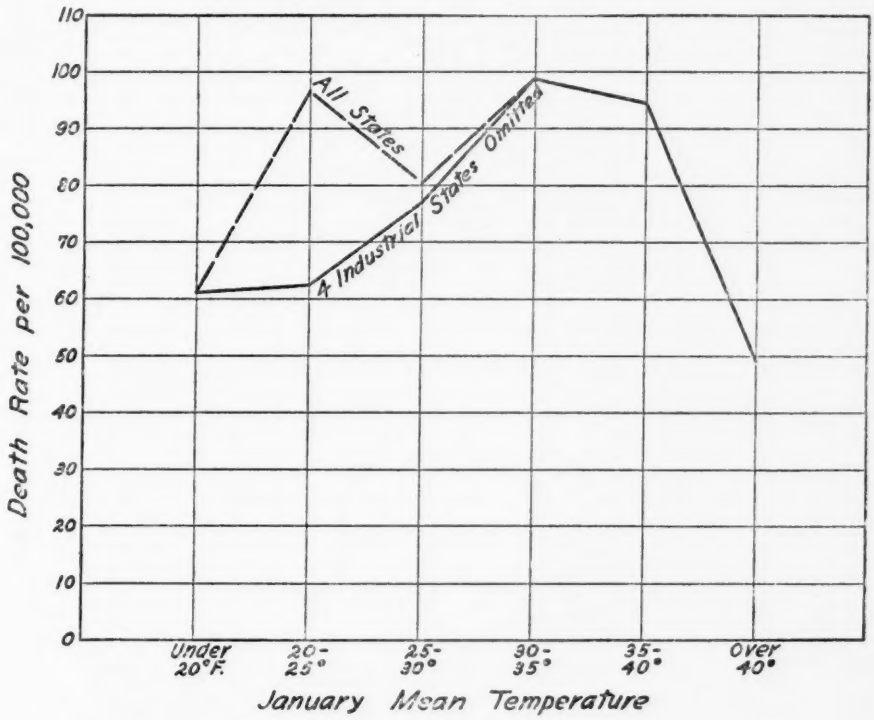

FIG. 1. WEIGHTED MEAN DEATH-RATES FOR 1920 FOR TUBERCULOSIS OF THE LUNGS IN UNITED STATES REGISTRATION STATES GROUPED ACCORDING TO JANUARY MEAN TEMPERATURE

The mean death-rates of the six groups of states show no such steady decline with increasing January temperature as would support the hypothesis that a severe winter climate or the poor ventilation of homes resulting from such a climate plays an important part in the etiology of tuberculosis. Instead of this, the states with winter temperatures below 20° or over 40°F. have lower death-rates than those with intermediate temperatures, taking the mean rates for all the states in the

TABLE 2

Adjusted death-rates per 100,000 for 1920 from tuberculosis of the lungs in United States registration states, grouped by January mean temperature

January mean temperature					
UNDER 20°F.	20° TO 25°F.	25° TO 30°F.	30° TO 35°F.	35° TO 40°F.	OVER 40°F.
Maine.....81.2	Massachusetts..91.8	Rhode Island..105.5	Delaware.....127.7	Virginia..... 89.7*	North Carolina..86.0*
New Hampshire..77.3	New York.....94.3*	Connecticut....100.8	Maryland..... 95.6*	Tennessee.....122.4*	South Carolina..66.4*
Vermont.....62.6	Michigan..... 70.6	New Jersey.... 98.3	Kentucky.....120.8*	Oregon..... 69.4	Florida.....60.3
Wisconsin.....72.9		Pennsylvania... 84.2*	Missouri.... .. 93.4		Mississippi.....50.7*
Minnesota.....75.6		Ohio..... 84.2	Washington.... 77.4		Louisiana.....79.8*
Nebraska.....35.1		Indiana..... 89.7			
Montana.....60.1		Illinois..... 85.1			
		Kansas..... 40.4			
		Utah..... 33.5			
Weighted mean death rate					
67.6	88.9	84.3	99.3	100.5	72.6† 65.6‡

* For whites.

† Including North Carolina.

‡ Not including North Carolina.

table. At its face value this would seem to be a direct reversal of the usual relation of an organism to its environment, where there is a certain optimum condition of any environmental element such as temperature in which the organism thrives best, while any departure in either direction from this optimum has an unfavorable effect. It is difficult, however, to see how such a reversal would come about. If the four predominantly industrial populations are omitted the mean rates increase steadily to the 30°–35° group and drop sharply only in the "over 40°" group.

As has already been stated, the data for adjusting the rural death-rates for sex and age are not available. Adjusted death-rates for each registration state as a whole and for white and colored in those states in which the colored population in 1920 was at least 200,000 are found in table IV of *Mortality Rates, 1910–1920* (3). These adjusted rates, for whites where available and in other cases for total population, are given in table 2, together with weighted mean death-rates for each temperature group. They show substantially the same features as the unadjusted rural death-rates of table 1. Evidently, differences in age and sex constitution of the populations of the different climatic zones are not the explanation of the differences in death-rate. In both tables the rates for some of the western states, especially Utah, Nebraska, and Kansas, are unusually low.

As the urban death-rates for tuberculosis of the lungs are higher than the rural, it might be supposed that the towns of 2,500 to 10,000 inhabitants have higher rates than the more strictly rural communities and that the higher tuberculosis rates in the middle sections of the country are due to a higher proportion of rural population being in these small towns. However, a spot diagram of the different states, correlating percentage of rural population in towns of 2,500 to 10,000 inhabitants with death-rates from tuberculosis, shows little or no correlation if Massachusetts and Rhode Island are excluded. These states, with 71 per cent and 85 per cent, respectively, of their rural inhabitants in small towns have both high crude rural tuberculosis death-rates, but from a comparison with the adjusted rates of table 2 it seems likely that much of this is a matter of age distribution and of industrial hazard.

Whether the change in tuberculosis death-rates with changing winter temperature is a true causal relation or due to some other factor, it is evident that it does not explain the difference in tuberculosis rates between Sweden and England, since all of Sweden and all of England except the extreme southwest have January temperatures well below 40°F.

SUMMARY

The white death-rate from pulmonary tuberculosis in the United States registration states is found to be higher in those states with a mean January temperature between 30° and 40°F. than in colder or warmer regions. Possible factors affecting this result are discussed. No evidence is found to support the view that severe winter climates, or poor ventilation resulting from them, are important factors in the etiology of tuberculosis.

The author wishes to express his indebtedness to Dr. Raymond Pearl and Dr. L. J. Reed for their counsel on various points in this paper.

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INHERITED IMMUNITY IN TUBERCULOSIS

H. G. CARTER[1]

We have for seven years now noted the interesting facts revealed each year by our summary tables showing the better results obtained in the mulatto Negro than in the black in the treatment of tuberculosis. We have recorded our results in previous articles (1) (2), but in each of these we stated facts and drew no definite conclusions as to the probable reason for these better results. Is it to be accounted for through accidental environment or through inherited immunity?

The American Indian and the American Negro offer an opportunity for important study in immunology. Here we have two races whose mortality rates, when infected with tuberculosis, are generally considered higher than those of their white neighbors.

We have at Piedmont Sanatorium made a careful observation of results shown by the Negro under treatment for pulmonary tuberculosis, comparing these results with those obtained in the two Virginia sanatoria admitting whites. The three institutions are operated under the same general management, and treatment, etc., is as nearly uniform as it is possible to make at three separate institutions.

We have also for seven years, as stated above, made a comparative study of the difference in results obtained from treatment of the mulatto and the black Negro in our institution. A careful study of these tables throws some interesting light on comparative resistance.

We find, in comparing the results obtained at the three institutions, that the number of cases improved at Piedmont Sanatorium for the colored is persistently less in every class, minimal, moderately advanced and far advanced. These results are comparatively shown in table 1 and chart 1, which show the percentage of each class admitted to each institution and the percentage improved under each class.

It will be noted from table 1 and chart 1 that there is a rather wide difference in percentage of the three classes admitted, minimal, moderately advanced and far advanced, but this difference is more marked between Catawba (white) and Blue Ridge (white) than it is between

[1] Piedmont Sanatorium, Burkeville, Virginia.

Catawba (white) and Piedmont (colored). Yet the results in percentage improved are about the same in the two white institutions and markedly less in the colored. This is especially true of the far-advanced class.

This apparently means less resistance on the part of the colored race. Yet it might be said that this apparently better resistance is due to better environment; that the white man on account of his better environment received less massive infection, and that he was slowly immunized through small infections. This is probably true, and might partly account for the difference. But it might also be said that during the short period of treatment the early cases of the colored race, placed under better environment, better food, etc., than those to which they are accustomed, should show certainly as great improvement as their

TABLE 1

Catawba (white): Minimal, 18 per cent of total and 92 per cent improved	
Blue Ridge (white): Minimal, 33 per cent of total and 91 per cent improved	
Piedmont (colored): Minimal, 12 per cent of total and 85 per cent improved	
Catawba (white): Moderately advanced, 22 per cent of total and 85 per cent improved	
Blue Ridge (white): Moderately advanced, 30 per cent of total and 84 per cent improved	
Piedmont (colored): Moderately advanced, 25 per cent of total and 71 per cent improved	
Catawba (white): Far advanced, 60 per cent of total and 60 per cent improved	
Blue Ridge (white): Far advanced, 37 per cent of total and 55 per cent improved	
Piedmont (colored): Far advanced, 68 per cent of total and 29 per cent improved	

white neighbors for whom the change in environment is not so marked. Then we have, on the one hand, a class of patients, probably more heavily infected from insanitary environment, changed to a much better environment and, on the other hand, a class of patients, probably less heavily infected or more gradually infected on account of more satisfactory environment, changed to an environment not so markedly different from that to which they are accustomed. The latter show more marked improvement under treatment.

We have in our institution kept a careful record of results as shown between the yellow mulatto and the black. It is my own experience that environment here is about the same. I sent personal letters to social service workers in every city in the state asking their opinion regarding the environment of the mulatto and black. Three thought

the mulatto to be better situated; four thought the black to be better; four thought both to be the same. One thought the mulatto more ambitious but less coöperative, and the black more coöperative and more likely to follow suggestions. These opinions from those in intimate contact with the home surroundings are about evenly divided regarding the environment of the two.

Table 2 and chart 2 show a comparison of results obtained in the treatment of white, mulatto and black. In order that the results will not be influenced by the larger percentage of early cases in the white

TABLE 2

White: Minimal, 24 per cent of total, improved 92 per cent
Mulatto: Minimal, 11 per cent of total, improved 91 per cent
Black: Minimal, 12 per cent of total, improved 82 per cent

White: Moderately advanced, 13 per cent of total, improved 84 per cent
Mulatto: Moderately advanced, 24 per cent of total, improved 79 per cent
Black: Moderately advanced, 21 per cent of total, improved 70 per cent

White: Far advanced, 53 per cent of total, improved 57 per cent
Mulatto: Far advanced, 65 per cent of total, improved 23 per cent
Black: Far advanced, 67 per cent of total, improved 20 per cent

TABLE 3

Average age of patients discharged from sanatoria

	1922	1923	1924	1925
Catawba	29.2	30.0	32.0	32.0
Blue Ridge	29.1	28.0	32.0	32.0
Piedmont	27.5	27.0	27.0	27.5

institutions, we have shown the percentage of improvements under each class separately. It will be noted that the best results are obtained in the whites, with the mulatto a close second; there is almost as great a difference between the results obtained in the mulatto and black as there is between those in the white and mulatto. The greatest difference in results is in the far-advanced class, in which we find a comparatively small percentage of improvements in both the mulatto and black.

A comparison of the average age of patients discharged from the three institutions, excluding those under ten years of age, shows the

patients discharged from the institution for colored to be less than the other two. This is shown in table 3.

CHART 1

Showing comparative results in three institutions, Catawba for white, Blue Ridge for white and Piedmont for colored.

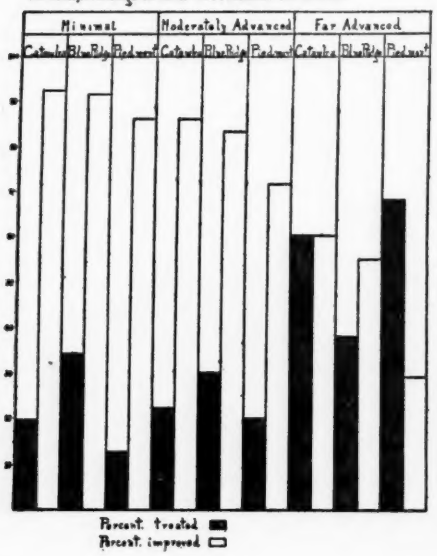


CHART 2

Showing comparative results of White, Mulatto and Black.

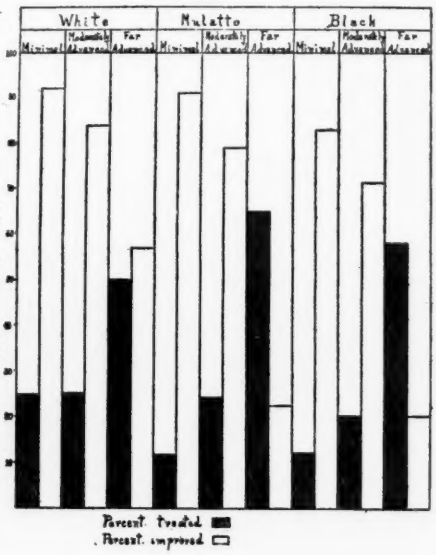


CHART 3

Showing average age of patients at three institutions.

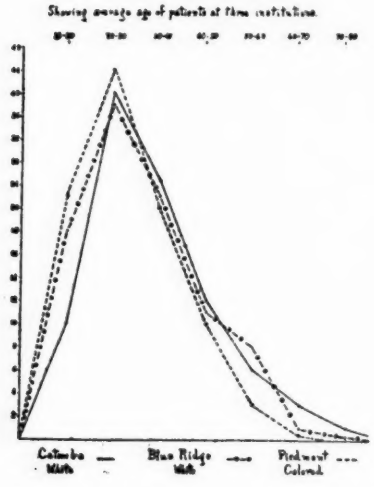
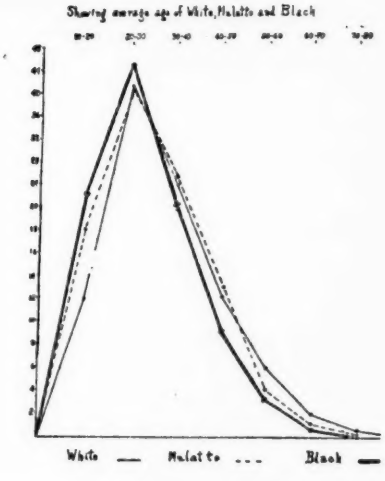


CHART 4

Showing average age of White, Mulatto and Black



We find the same fact revealed in a comparison of ages of those who died in the institutions as shown in table 4. This could mean either

lessened resistance on part of host or more massive infection from less satisfactory environment. But a comparison of the average age of the mulatto and the black (table 5) in our institution shows the black to be several years younger than the mulatto. This seems again to rule out environment as the sole factor.

A graphic curve showing the number treated at each institution between 10 and 20, 20 and 30, etc., shows all three institutions to reach a maximum between twenty and thirty years of age. The curve representing Piedmont Sanatorium is highest at 10–20 and 20–30 and below the other institutions from 30 to 80. The infection has become evident in the host at an earlier age and there are a smaller percentage that survive to middle and old age.

TABLE 4

Average age of patients at death

	1922	1923	1924	1925
Catawba	33.5	32.0	28.0	28.0
Blue Ridge	33.2	31.7	31.0	31.0
Piedmont	26.5	26.0	29.0	27.0

TABLE 5

Average age of mulatto and black

Average of black	26.0
Average of mulatto	28.7
Average age at death: black	26.7
Average age at death: mulatto	28.2

The same curves representing the white, mulatto and black show the black to be the highest prior to the age of thirty and lowest after thirty. The white is lowest before thirty and highest after thirty. The mulatto is between the two, and more nearly approaches the white line than the black line. This apparently means greatest resistance on the part of the white and least on the part of the black, the mulatto occupying a middle ground.

Brownlee, in a survey of the British Isles some years ago, found the average age at death from tuberculosis highest in those thickly settled regions where infection was greatest, and lowest in the rural districts where infection was least. We find the average age of those who have developed definite tuberculosis, as well as those who have died of the

disease, to be the highest in the white people and lowest in the black Negro; the mulatto shows an average between the two. Brownlee draws the conclusions that resistance to the disease is increased by contact, there being less of the "young adult type" in those districts most heavily infected. Our records seem to show yet another factor. The percentage of rural and urban population is about the same in the three institutions, also in the black and mulatto, yet we have a greater percentage of "young adult type" in the black than we have in the mulatto, and a greater percentage of this type in the mulatto than in the white. Our conclusions are that the difference is due mainly to inherited resistance.

SUMMARY AND CONCLUSIONS

In a comparison and study of some 5,000 white patients and 1,700 colored patients treated under the same conditions, we find results obtained in the whites persistently better than results shown for the colored. Carrying this further, we find that results shown in the mulatto Negro are better than those shown in the black, but not so good as results in the white. We find that the average age of those treated is greatest in the white and least in the black, while the mulatto occupies a middle ground. The same is true of the average age at death, namely, highest in the white and lowest in the black. We find the greatest incidence of tuberculosis under thirty years of age in the black, next in the mulatto and least in the white. Over thirty this order is reversed.

It might be said that environment accounts for the difference between the two races; this however cannot be said of the mulatto and black. My conclusions are that this better resistance in both instances is due to an inherited immunity from an ancestry that has accumulated that immunity through contact with tuberculosis. The white race transmitting to the mulatto a part of a stronger immunity accumulated through longer contact, this transmitted immunity plus that acquired by the mulatto gives him a better resistance to tuberculosis than the black, whose immunity has been accumulated entirely by him since being in contact with civilization, and almost entirely in the last seventy-five years since freedom has thrown him in contact with crowded centres of population.

RESISTANCE TO TUBERCULOUS INFECTION OF GUINEA PIGS RENDERED SKIN SENSITIVE WITH DEAD TUBERCLE BACILLI[1]

L. DIENES AND E. W. SCHOENHEIT

The relationship between local hypersensitiveness, resistance to infection and generalization of disease has been a matter of interest since it has been clearly established that a considerable degree of hypersensitiveness can be produced with dead tubercle bacilli (1) (2) (3). The hypersensitiveness so produced does not seem to differ from the hypersensitiveness developing after the infection (4); only it is usually considerably less in degree,—according to the estimation of Langer (5) about one-tenth to one-hundredth as great. Krause (6) attributes to local hypersensitiveness a large part of resistance to tuberculosis, and expresses the opinion that the difference in the sensitizing effect of living and non-living bacteria is quantitative rather than qualitative. Zinsser (7) and his associates have given experimental evidence of an increased resistance to infection of guinea pigs made hypersensitive by injections of killed tubercle bacilli. A connection between the resistance and hypersensitiveness is not generally accepted; Loewenstein (8), one of those who does not accept it, bases his views mainly on the experience with bovovaccination. We do not feel that we are in a position to discuss how far the evidence offered by bovovaccination is decisive. An absence of reaction to the subcutaneous administration of tuberculin after bovovaccination has been generally found. The same observation was made by Calmette after intravenous administration of his *BCG* bacilli (9), and after subcutaneous administration of these bacilli a very high grade of resistance was found to exist several months after skin sensitiveness to tuberculin disappeared (10). Notwithstanding this, Calmette believes in the importance of hypersensitiveness for resistance. There is nothing mentioned in Calmette's publications about the skin sensitiveness of guinea pigs immunized with intracardial injections of *BCG* bacilli (11). Selter

[1] From the Von Ruck Research Laboratory for Tuberculosis, Asheville, North Carolina.

TABLE 1

NUMBER OF EXPERIMENT	TREATMENT OF GUINEA PIGS	SKIN SENSITIVENESS (0.01 CC. WATERY EXTRACT INTRACUTANEOUSLY)	MODE OF INFECTION	RESULTS
1	12 guinea pigs injected 3 times at weekly intervals, each time with 3 mgm. dry material HD defatted emulsion, intracardially.	No reaction found	5 weeks after last injection 3 guinea pigs were infected intracardially with 0.025 mgm., 2 with 0.05 mgm. HD emulsion, wet weight. 3 guinea pigs were infected subcutaneously with 0.025 mgm., 2 with 0.05 mgm. HD emulsion. 10 control guinea pigs of the same size were infected in the same way as the treated guinea pigs.	The treated animals infected intracardially died on an average in 19 days. The liver was very much enlarged, the weight of spleen was on the average 7.5 gm. The control animals infected intracardially died on the average in 25 days. Lungs, and in some animals the liver, were full of miliary tubercles. Weight of spleen on the average 8 gm. The subcutaneously infected animals were killed 33 days after the infection. All were found with generalized tuberculosis. Average weight of spleen in the treated animals was 10.5 gm., in the controls 8.6 gm.

2	At 2 weeks' intervals intracardially 0.1, 0.7, 1.5, 3.0 mgm.; in all 5.3 mgm. dry material HD defatted emulsion. (9 guinea pigs.)	2 of 9 animals have shown a slight skin test, examined 10, 16, and 40 days after the last injection.	6 weeks after the last injection infected partly with 0.025 and partly with 0.1 mgm. of HD emulsion, wet weight.	In all animals of series 2 to 4 and the controls killed 2 months after the infection, the infection reached the spleen, and, with the exception of 1 control infected with 0.025 mgm. reached the lungs. With the smaller infection dose the advance of the disease was visibly less in the controls than in the treated animals. The average weight of the spleen is 5.2 gm. in the controls, against 6 gm. in series 3 and 4, and 4 gm. in series 2.
3	Every 3rd day, 6 times 0.2 mgm., 6 times 0.4, and 6 times 1 mgm. intracutaneously; 9.6 mgm. dry material in all. (8 guinea pigs.)	6 weeks after last injection in 3 guinea pigs strong skin reaction, in 4 moderately strong, and in 1 a slight reaction was found. 10 weeks after last injection 1 formerly strong reaction was changed to moderate.	10 weeks after the last injection infected with the same bacillus emulsion and in the same manner as series 2	
4	Treated with the same amount, given at the same intervals as series 3, but subcutaneously. (6 guinea pigs.)	6 weeks after last injection 3 strong and 3 moderately strong reactions were found. 10 weeks after last injection 1 strong reaction was changed to moderate.	The same as series 3. Together with the 3 last series 18 control guinea pigs were infected.	

(12), who formerly advocated the significance of hypersensitiveness for immunity, later turned against this conception.

In the case of typhoid fever skin sensitiveness seems to be a sign of infection, and not of resistance (13). MacKenzie and Shutai (14) found no connection between skin sensitiveness and resistance in the case of pneumococcus infection.

The experience obtained with other diseases naturally permits of no direct conclusion in connection with tuberculosis, but we should not forget that the connection between hypersensitiveness and resistance in the case of tuberculosis was presumed mainly from the common occurrence of them in infected animals and the absence of both in animals treated with dissociated bacterial products.

We describe in this paper an experiment with four series of animals, which originally was planned for another purpose. Because of the negative outcome of these experiments we do not deny the significance of hypersensitiveness in immunity, but call attention to the need of close study of the conditions under which the two phenomena occur, and the need of quantitative determinations of both sensitiveness and of degree of resistance in experiments.

Two series of guinea pigs were treated with intracardial injections of a bacillus emulsion; two series with ascending doses of the same emulsion given intracutaneously and subcutaneously respectively. The treatment of the animals, the resulting skin sensitiveness, the mode of infection, and the results of the experiment are described in table 1. The emulsion used for the treatment was one of the moderately virulent strain *HD*. The living bacillary mass was dried over sulphuric acid, then extracted during two days with anhydrous ether, and during one day with absolute alcohol, and ground three days with distilled water with the addition of some chloroform. We have on several occasions found that the bacteria are killed by this treatment, and two guinea pigs injected with 20 mgm. of dry material of this emulsion (the injection was distributed in several places) did not develop disease, and their spleens were not infectious for other guinea pigs. In series 3 and 4 the time for infection was selected quite long after treatment, because previous experience had shown that guinea pigs similarly infected four weeks after treatment did not show increased resistance against the infection.

Thirty-three days and two months respectively after infection the guinea pigs were killed. Autopsy showed marked individual differences

in the progress of the disease, but no uniform difference could be found between the controls and either the intracardially treated guinea pigs, which did not show appreciable skin sensitiveness, or the intracutaneously and subcutaneously treated guinea pigs with marked skin sensitiveness. The average weight of spleen is given as an illustration of the quite uniform spreading of the disease in the treated and in the control animals. In the individual animals the weights of the spleens varied between 0.5 and 31 gm. Of the tubercle-bacillus culture used for the injection 0.025 mgm. killed the guinea pigs in about $2\frac{1}{2}$ to $3\frac{1}{2}$ months. During the experiment the animals were in a very good state of nutrition and there was no epidemic among them.

There are several features of the experiment which have to be taken into consideration in interpreting the results. Is the emulsion used equivalent, as an antigen, to the heat-killed bacteria? We did not compare them systematically, but our bacterial material with a single subcutaneous injection of sufficient amount has produced a strong hypersensitiveness in guinea pigs. The skin sensitiveness was not titrated, although certainly it was considerably less than is generally found in moderately advanced infections. The animals were infected five, six and ten weeks, respectively, after the injection. In ten weeks the resistance eventually begins to fade, but, as stated above, in similarly treated guinea pigs, in which we did not follow the skin sensitiveness, we did not find increased resistance four weeks after the end of the treatment. The killing of the guinea pigs after a certain time seems to us to give sufficient information about the spreading of the disease, and even it is perhaps less easily influenced by secondary circumstances as the time of the survival is affected. We think we can take into consideration only pronounced differences between the treated and the control animals. The latter consideration caused us to decide not to use a larger number of animals.

The most serious objection against any conclusion drawn from this experiment is that the skin sensitiveness which we have produced is considerably less than the skin sensitiveness of infected animals. The same dose of watery extract which caused about 20 mm. of white and red swelling in the six most sensitive guinea pigs caused necrosis in the infected animals, and a reaction similar to that observed in our treated animals is caused by five to ten times less extract in the infected animals. However, the skin sensitiveness of our most sensitive animals was probably near to the sensitiveness described by Petroff. In the guinea pigs made resistant with a slight infection the skin sensitiveness

is not always found considerably higher than in our animals, and the fact that we could find no difference between the animals with and without skin sensitiveness shows that the elevation of resistance which is perhaps connected with the lower degree of skin sensitiveness can be so low as not to show itself with a moderately severe experimental infection. The resistance of immunized animals is usually found less against a natural than against even a severe artificial infection.

It is significant that injections into the circulation, which are far superior for the production of antibodies, are practically without effect for the production of skin sensitiveness. In an experiment, which unfortunately was interrupted by an epidemic among the guinea pigs, we injected daily 0.002 mgm., dry weight, killed bacteria subcutaneously always in the same place. Notwithstanding that a considerable hard tumor was produced we found only 3 of 15 animals to have a weak skin hypersensitiveness after six weeks. The noneffectiveness of the intracardial injections emphasizes the significance of a persistent local focus, and the failure of the daily injection of small doses the importance of a sufficient dose. There is a significant contrast between the large amount of killed bacteria needed for the production of hypersensitiveness and the minute amount of bacteria present in the organs at the time of the appearance of the hypersensitiveness in the infected animals.

CONCLUSIONS

1. Intracardial injections of killed tubercle bacilli were practically without effect in producing skin sensitiveness in guinea pigs.

2. Guinea pigs rendered tuberculin-sensitive by intracutaneous or subcutaneous injections of killed tubercle bacilli did not show in our experiment higher resistance against the infection than did the control animals.

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EDITORIAL

THE DECLINE OF TUBERCULOSIS MORTALITY IN THE UNITED STATES AND THE INFLUENCE OF THE INFLUENZA EPIDEMIC OF 1918

In 1900 the population of Continental United States was 76,129,408, and the percentage of population in the Death Registration Area was 40.5. The reported deaths from tuberculosis in this Area of only two-fifths of the population numbered 62,096. By a simple calculation we therefore arrive at 153,781 as the total tuberculosis mortality for the United States in 1900, a figure which, while in no way exact, is sufficient, and no doubt as close to the actual total of deaths from tuberculosis as can be obtained.

A quarter-century has since passed. By 1925 the Registration Area had grown to 88.4 per cent of the population, which for the same year may be estimated at 113,513,720 souls, or almost forty millions more than in 1900. While the death-rate for tuberculosis for the year cannot yet be calculated, on early and incomplete returns it is estimated to have been 88 per 100,000 inhabitants. Whatever it may turn out to be, 88 cannot be far wrong, and we may therefore calculate a mortality of 99,892 for 1925. In other words, in 1925 less than two-thirds as many people died of tuberculosis as in 1900 in the United States, and this in a population over half again as large as in 1900. Our citizens' chances against tuberculosis to-day are almost two and a half times better than they were in 1900, or as 88 is to 202. The actual diminution of mortality is more than fifty thousand from a total of over 150,000. We begin now to gain some appreciation of the "push" and power behind what we designate as the *decline of tuberculosis*.

In terms of lives actually delivered from tuberculosis the decline has been staggering in its proportions, and no doubt momentous in its influence on the natural history of American mankind,—on life and death in general, on human purpose and accomplishment, on national endeavor and achievement. If, every year since 1900, 202 of every 100,000 people had died of tuberculosis, as they then did, the accumulated mortality for the disease would have been 4,856,251 for the next twenty-five

years. As a matter of fact, it was nearer 3,479,535. The difference is 1,376,716, a figure which we may regard as the number of lives "saved"

Approximate Mortality from Tuberculosis, All Forms, in United States, Since 1900, Compared with Expected Mortality - if Death Rate of 1900 had continued throughout the Period

Year	Population of United States	% of Pop. in Reg Area	Deaths in Registration Area	Death Rate in Reg. Area	Deaths in United States*	Deaths at Rate of 1900	Estimated "Saving" of Lives
1900	76,129,408	40.5	62,096	202	153,781	153,781	
1901	77,747,402	40.3	61,767	197	153,162	157,050	3,888
1902	79,365,396	40.4	59,083	185	146,826	160,318	13,492
1903	80,983,390	40.4	61,640	189	153,059	163,586	10,527
1904	82,601,384	40.4	66,921	201	166,029	166,854	826
1905	84,219,378	40.4	65,478	192	161,701	170,123	8,422
1906	85,837,372	48.9	75,648	180	154,507	173,391	18,884
1907	87,455,366	49.2	76,759	179	156,545	176,660	20,115
1908	89,073,360	52.5	78,409	168	149,643	179,928	30,285
1909	90,691,354	56.1	81,835	161	146,013	183,196	37,183
1910	92,267,080	58.3	86,309	160	147,627	186,379	38,752
			Totals, 1901-1910 =		1,535,112	1,717,486	182,374
1911	93,682,189	63.2	94,205	159	148,955	189,238	40,283
1912	95,097,298	63.5	90,360	150	142,646	192,097	49,451
1913	96,512,407	65.5	93,421	148	142,838	194,955	52,117
1914	97,927,516	67.2	96,903	147	143,953	197,813	53,860
1915	99,342,625	67.5	98,194	146	145,040	200,672	55,632
1916	100,757,735	70.8	101,396	142	143,076	203,531	60,455
1917	102,172,845	73.4	110,285	147	150,194	206,389	56,195
1918	103,587,955	78.5	122,249	150	155,381	209,247	53,866
1919	105,003,065	81.1	106,985	126	132,304	212,106	79,802
1920	106,418,175	82.2	99,916	114	121,317	214,965	93,648
			Totals, 1911-1920 =		1,425,704	2,021,013	595,309
1921	107,833,284	82.2	88,135	99	106,755	217,823	111,068
1922	109,248,393	85.3	90,452	97	105,941	220,652	114,711
1923	110,663,502	87.6	90,732	94	104,024	223,541	119,517
1924	112,098,611	88.4	89,724	91	102,107	226,439	124,332
1925	113,513,720			88	99,892	229,297	129,405
			Totals, 1921-1925 =		518,719	1,117,752	599,033
			Totals, 1901-1925 =		3,479,535	4,856,251	1,376,716
The National Tuberculosis Association was Founded in 1904							
The First Great Influenza Epidemic Occurred in 1918							
			Totals, 1904-1918 =		2,254,148	2,830,474	576,326
			Totals, 1919-1925 =		772,340	1,544,823	772,483

*Estimated on Basis of Rate for Registration Area

from tuberculosis since 1900, through whatever influences have since been operating to cut down the disease.

In the accompanying table are set down, year by year, (1) the number

of deaths from tuberculosis actually reported from the Registration Area, (2) the estimated number for the whole United States (Continental), (3) the "expected" number if the rate of 1900 had continued until the present time, and (4) the estimated saving of lives, as figured on the difference between the deaths that occurred and those that would have occurred at a continuing rate of 1900. The table shows that during the first decade of the century the "saving" of lives was 182,374, and during the second decade 595,309. For the first twenty years it was therefore 777,683.

But in the five years since 1920 the number of lives "saved," 599,033, is not so far away from the total for the twenty years before. Roughly, the yearly average for 1921–1925 is 120,000; for 1911–1920 it is 60,000; and for 1901–1910, 20,000. Accordingly, the combined factors of diminishing death-rate and increasing population are, during the present decade, being responsible for an annual average of twice as many lives "snatched" from tuberculosis as in the previous decade, and six times as many as in the first decade of the century. Among the people consumption is undergoing an enormous dilution; so remarkable indeed as to have become noticeable to even the ordinary citizen with no particular interest in the subject. More than one person of sixty years and upward has asked the writer what has become of consumption: in early life he knew it as the most common serious malady among his acquaintances; to-day he meets with it little or not at all. The answer no doubt is that such a man is living in a United States with consumption certainly not more than one-quarter as prevalent as in 1880.

As has been frequently noted by many observers, the year 1918 marks a sharp dividing point in the curve of tuberculosis mortality of the quarter-century. In the eighteen years before and including 1918 the rate declined approximately 25 per cent, and in the seven years since 1918 approximately 41 per cent. On the basis of a continuing rate of 1900 the number of lives "saved" between 1901 and 1918 may be calculated at 504,233, and for the seven years since 1918 at 772,483; or at a yearly average of roughly 33,500 for the first eighteen years and of 110,000 for the last seven years of the period under consideration. So sharp a break in the mortality curve is altogether extraordinary, and its explanation presents to the investigator the real problem of the more recent epidemiology of tuberculosis.

The accelerated decline has frequently been attributed to the influenza pandemic of 1918. In the United States the first, and by all odds the most virulent, of several waves of influenza raged throughout the autumn of that year. Beginning along the Atlantic seaboard in Septem-

ber, by the end of the year it had reached its peak everywhere in the nation and subsided. No accurate figures of the mortality of this epidemic have ever been published, but conservative estimates by experts have placed its deaths at about five hundred thousand for Continental United States.

As soon as it was found that the annual curve for tuberculosis mortality for 1919 was without its customary late winter and early spring peak, and that the death-rate for the year had made the enormous fall of 16 per cent from that of 1918 (126 for 1919 as against 150 for 1918), it was almost generally assumed that influenza in 1918 had profoundly affected tuberculosis mortality by causing the deaths of a great number of consumptives and thus removing these from the tuberculosis mortality returns of 1919 (and perhaps later), where they would have been found in an ordinary course of events. This opinion was further strengthened when it transpired that the tuberculosis death-rate for 1920 had shot down to 114 or another 10 per cent from that of 1919, and when for 1921 the rate stood at 99 or a further decline of 15 per cent within a single year. It is doubtful whether the entire history of disease can match so extraordinary and sensational an event—a perennial malady so deeply rooted, firmly entrenched and universally diffused as tuberculosis undergoing a reduction of mortality of one-third within three years. And during the succeeding four years the reduction has been about a further 12 per cent.

With the passage of time it has become more and more difficult to believe that the influenza of 1918 has *directly* been responsible for the unexampled assuagement of tuberculosis since then,—*directly*, in the sense that by taking off thousands with tuberculosis at one stroke and at one time it depleted the tuberculosis mortality returns of the succeeding few years, in which these thousands must ordinarily have been included. An examination of vital statistics makes it almost impossible to entertain such an opinion.

For instance, before 1918 the "expected" tuberculosis mortality for the month of October in New York State was annually about 1,000 deaths. For October, 1917, the actual number was 1,089. The same month in 1918 exhibited a sharp peak of tuberculosis mortality in New York State, which was exactly superimposable upon the curve of influenza mortality; and for the month there were reported 1,520 deaths from tuberculosis, or an increase of almost 50 per cent over the expected. In October, 1919, there were only 813 tuberculosis deaths, and in October, 1920, 726 deaths.

It is quite likely that every one of the "excess" tuberculosis deaths during October, 1918, was due to influenza in a consumptive patient. If so, then influenza removed 431 persons who ordinarily might have contributed to the tuberculosis mortality of later years. Very roughly, one-tenth of the nation's population dwells in New York State. It is certain that, as regards influenza, the situation for the country at large was no worse than in New York. If then we use events in New York State as our gauge, we may multiply the latter's excess tuberculosis mortality by 10, and arrive at 4,310 persons with consumption as having died of influenza throughout the United States during the great epidemic of 1918. And even though we grant that the number was larger, we cannot imagine it to have been so great as to account (by removing consumptives) for upward of 23,000 fewer tuberculosis deaths in 1919 than in 1918, 34,000 fewer deaths in 1920 and 49,000 in 1921, or a total of 106,000 below the figures for 1918 (155,381) within the three years afterward. (See accompanying table.)

Another way of viewing the possible effects of the influenza epidemic on tuberculosis is to ask whether it may not have taken off thousands of persons without active tuberculosis or in minor stages of activity at the time, who later might have developed the disease and died of it. Such an idea gains in plausibility when we consider that one notable feature of the epidemic was its excessive mortality among the younger and more vigorous elements of the population, from about ages 18 to 40, a period also highly prone to the development of pulmonary tuberculosis.

We may examine this possibility by estimating the accumulated mortality from tuberculosis on the basis of a continuing rate of 1918, and determining the difference between this total and the number of those actually dead from tuberculosis since 1918. The calculations result as follows:

YEAR	ACTUAL NUMBER OF DEATHS FROM TUBERCULOSIS	DEATHS AT TUBERCULOSIS RATE (150) OF 1918	DIFFERENCE
1919	132,304	157,505	25,201
1920	121,317	159,627	38,310
1921	106,755	161,750	54,995
1922	105,941	163,873	57,932
1923	104,024	165,995	61,971
1924	102,107	168,148	66,041
1925	99,892	170,271	70,379
Totals.....	772,340	1,147,169	374,829

We find that since 1918 there have died from tuberculosis about 375,000 fewer persons than would have been the case had the rate of 1918 continued. Such a difference is altogether too great to imagine it being due to potential or active consumptives falling prey to influenza, of which the combined excess mortality of all successive waves since 1918 (and including the latter) can hardly be more than 600,000. The presumption would have to make more than one-half of all influenza victims tuberculosis patients or "prospects," and such a contingency is preposterous.

The writer regards an influence of influenza on tuberculosis as incontestable, but with the passage of time he is inclined to minimize its direct effect (though this was appreciable), and to consider it as one of several contributing factors which conceivably began to converge at or about the same time. For many years the conditions of living in the United States were of a kind favorable to a diminishing incidence of tuberculosis. They are familiar to every one and need not be enumerated anew. As time went on they became enhanced, and with the War, and since, went to quite unexampled heights. Operating alone, they would have continued to reduce tuberculosis mortality since 1918, though not at the rate which has actually been in effect.

To their aid, and markedly so, came sanatorium development, and organized effort. These did not culminate in 1919; they were then just getting into their stride. Up to 1915 the sanatorium era in the United States was one largely of preparation; it has since been one of development and perfection. Before 1915 a Christmas Seal sale of as much as a million dollars was beyond our rosiest dreams; since then we have been looking for three, four, and now five million dollars a year.

And then along came an epidemic, or, rather, a series of epidemics, of acute respiratory diseases, which removed, quite out of place and beforehand, a not inconsiderable block of infectious foci. If we examine the figures, we may not make this too large. It was no doubt responsible for an increase of 3 points over the rate of 147 for 1917, to 150 for 1918, just as the increase of pneumonia (influenza?), becoming noticeable in 1917, may have sent the tuberculosis rate of 1917 to 147 from 142 for 1916. Between them, the years 1917 and 1918, both with excessive pneumonia rates, took about 12,000 more deaths from tuberculosis than the year 1916 had promised. The actual number of the tuberculous removed by influenza-pneumonia was, no doubt, somewhat greater than that returned as died of influenza and pneumonia with tuberculosis. The effect of suddenly taking away a twentieth or a tenth of consumptives destined

to die one, two or five years later must be to bring down the expected death-rate and, for an indefinite time, lessen the number of new contacts, new cases, new deaths. So much the influenza and pneumonia of 1917 to 1923, and up to the present, have done; but, of itself, it would seem to be a minor part of what has happened to tuberculosis. Yet, in so doing, influenza and pneumonia may have played into the hands of broad socio-economic, and sanatorium and anti-tuberculosis-movement influences, and added to the effects of these considerably.

A. K. K.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

I. A NON-PROTEIN MEDIUM SUITABLE FOR THE PRODUCTION OF TUBERCULIN IN LARGE QUANTITY[1,2]

ESMOND R. LONG AND FLORENCE B. SEIBERT

The results reported in the following series of articles are the outgrowth of a study on the nutrition of the tubercle bacillus which has been in progress for several years. These results were presented in brief at a recent meeting of the National Tuberculosis Association (1), and have been published in a preliminary article (2), in which experimental details were omitted. The experiments and conclusions drawn from them are given in full in the following pages.

One of the results of this study on nutrition has been the evolvement of a nonprotein medium of known chemical composition, suitable for the production of tubercle bacilli in large quantities. Media of well-defined chemical composition have been used for the growth of tubercle bacilli by many investigators since Kühne's first experiments in this direction in 1894. Formulae for a dozen such media are given by Calmette (3). Many more than this number, however, have been used, various investigators claiming special advantages for different combinations. A review of the principles underlying the use of media of this type has been given by Long (4).

These media have much in common. All contain glycerol, a salt of phosphoric acid, nitrogen in simple form, and a variety of inorganic salts. According to Lockemann (5) the absolute essentials for growth of the tubercle bacillus are glycerol, a nitrogenous substance, phosphoric acid, potassium and magnesium. However, although very simple media may enable the tubercle bacillus to maintain its existence and multiply slowly, it is necessary to complicate the medium somewhat in order to secure luxuriant growth. This may be done without sacrificing chemical definity. It is not necessary to use substances of undefined chemical

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

composition, such as proteose or peptone. Nor does there appear to be any advantage in adding preparations of vitamines, which are of still less understood composition. A medium can be prepared which will permit growth equal to the maximum obtained in glycerolated beef bouillon, in which no substance is present for which a simple chemical formula cannot be written.

The following combination has been found to yield 100 to 150 gm. of moist bacilli to the litre in 4 to 6 weeks of growth (H37 or R1 strains of the Saranac Laboratory):

Asparagine	5
Ammonium citrate	5
Potassium acid phosphate	3
Sodium carbonate (anhydrous)	3
Sodium chloride	2
Magnesium sulphate	1
Ferric ammonium citrate	0.05
Glycerol	50
Water	1000

Three organic substances are present, namely, glycerol, citric acid and asparagine. Eight inorganic ions are present, four basic and four acidic, namely, ammonium, potassium, sodium, magnesium, chloride, phosphate, sulphate and carbonate. It will be noted that calcium is absent, nor does its presence appear either necessary or desirable. It is possible, of course, that the bacilli absorb traces from the glassware. By omitting calcium it is possible to avoid those calcium-phosphate precipitates which often cloud the usual media.

One of the principal advantages of this medium is its buffered state, due to its content of potassium acid phosphate, sodium carbonate and asparagine. No titration is necessary, the combination of the substances listed resulting in approximate neutrality. A considerable proportion of the acid and alkali evolved in the different phases of bacillary growth may be absorbed in this medium without marked change in reaction.

Another advantage lies in the presence of dissolved carbon dioxide. The acid phosphate and some of the sodium carbonate react, with the liberation of a certain amount of carbon dioxide, which is believed to accelerate growth of the tubercle bacillus, particularly, according to Wherry and Ervin (6), in concentrations corresponding to pressures between 17 and 47 mm. It should be noted, however, that Novy and Soule (7) did not confirm the work of Wherry and Ervin. Rockwell

and Highberger (8), on the other hand, believe carbon dioxide is a vital factor in the growth of the bacillus.

Polybasic acids have been found advantageous by numerous investigators. Of them all, citric acid has proved the most popular, although the extent and manner of its utilization by the tubercle bacillus are yet to be worked out. It is conveniently used as the ammonium salt, which is equally useful to the bacillus for its citric acid and ammonia.

French investigators have done much work in recent years on the enhancing effect of iron on plant growth, and Calmette and Massol (3), and later Sauton (9), have found a similar favorable influence on the growth of the tubercle bacillus. This has been confirmed by others. According to Sauton iron in a concentration of 1 to 100,000 is sufficient to triple the weight of growth. Henley (10) found that the addition of ferric sulphate in concentration of 20 mgm. per 100 cc. of medium added 41 per cent to the pellicle weight in the course of eight to twelve weeks of growth.

In our experience iron has been found useful, but not as valuable as Sauton claimed. In 1 to 20,000 concentration of the ferric ammonium citrate it increases the yield to some extent, and therefore is used routinely in the medium we recommend. In a typical experiment in which the yield of bacilli on our medium with and without iron was determined, the following figures were obtained:

12 bottles of the complete medium, containing in all 420 cc., were inoculated on July 1, 1925, with R1, and the bacilli were filtered off on August 12, 1925, thoroughly washed and dried *in vacuo* over sulphuric acid. The yield of dry bacilli was 7.1 gm.

12 bottles of medium containing in all 420 cc. just like the above, except that the iron was omitted, were treated similarly. The yield of dry bacilli was 6.6 gm.

Asparagine has proved to be one of the most useful of all simple sources of nitrogen in synthetic media for bacterial culture, and has been a constituent of most of the synthetic media proposed for the cultivation of tubercle bacilli. The manner in which its molecule is attacked by the tubercle bacillus has been investigated by Long (11), who found the amide group particularly labile in the presence of the tubercle bacillus.

As asparagine is the most expensive constituent of our medium the possibility of leaving it out in the production of tubercle bacilli on a large scale was considered. With ammonium citrate, however, as the sole

source of nitrogen, the yield was greatly inferior, as the following figures show:

12 bottles of the complete medium, containing in all 420 cc., were inoculated on February 17, 1925, with R1, and the bacilli were filtered off on May 15, 1925, thoroughly washed and dried *in vacuo* over sulphuric acid. The yield of dry bacilli was 7.0 gm.

12 bottles of the same medium from which asparagine had been omitted, and in which the ammonium citrate had been increased to make up for the loss in nitrogen, were treated similarly. The yield of dry bacilli was 1.3 gm.

Tubercle bacilli grow on our medium in the form of a heavy pellicle. They can be easily separated by filtration and readily washed, and thus obtained in a mass which is nearly pure white. Washing with salt water or distilled water quickly removes the crystalline constituents of the medium, but prolonged washing on the filter is necessary to get rid of the glycerol. If the latter is not wholly removed it interferes with subsequent drying and leads to the production of a gummy preparation. If, on the other hand, the glycerol is thoroughly washed out, a dry, crumbly mass, which is only faintly yellowish, is secured on drying *in vacuo*.

Through the coöperation of the Research Committee of the National Tuberculosis Association, the H. K. Mulford Co. and Parke, Davis & Co., tubercle bacilli have been grown on this medium on a large scale. About 60 kgm. of moist bacilli were easily secured in a few months; these are being analyzed by Professor T. B. Johnson and his colleagues of Yale University.

The filtrate from these bacilli has been the subject of the experiments recorded in the articles which follow. Typical samples give all the usual protein tests and strong tuberculin reactions in tuberculous animals, and are quite nontoxic for normal animals.

There has been much discussion concerning the production of protein in synthetic media as a result of the growth of tubercle bacilli. This has been reviewed by Long (4). Frequently it has been found that highly active tuberculins failed altogether, even when concentrated, to give the simple biuret test. We have had the same experience ourselves, and one of us also has reported the production of an active tuberculin which appeared to be free from protein (12). We believe now that all such preparations contain protein, but that frequently this may be in too small quantity to detect by the usual means. Recently we (13) found one

cause for error. The presence of glycerol, which is a regular constituent of synthetic media, interferes strongly with the biuret reaction. However, most of the samples of tuberculin prepared on our medium give a good biuret test on concentration. In addition, the Millon, vanillin, glyoxylic, ninhydrin and Molisch tests are positive. Alcohol, acetic acid, tannic acid and ammonium sulphate all throw down a protein precipitate. The color of the tuberculin preparation is light yellowish-brown, and the odor is quite like that of tuberculin prepared on the usual peptone and meat-infusion media.

The chief advantage of this type of tuberculin preparation for chemical study lies in the ease with which substances produced in the medium by growth of the bacillus may be separated. As all constituents of the original medium are known, reagents may be employed which precipitate only the new substances. In the next article (study II) some of the methods used in the separation of material formed in the medium by or from the tubercle bacilli will be described.

Inasmuch as the appearance of protein in the medium and the development of tuberculin activity are coincident, the chief object of this investigation has been to determine the relation of this protein to the active principle of tuberculin. It is conceivable that the protein might be the active principle, or that several proteins might be present, one of which is the active principle; or, as several investigators have supposed, that a cleavage product of protein might be present and be the active principle; or, finally, that this principle might be something quite different from protein, separate from the protein of the medium entirely, or at the most merely adsorbed to it. As described in the following articles, methods of dialysis, adsorption, enzymic action and protein precipitation were used in the study of these possibilities.

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THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

II. PRECIPITATION WITH ACETIC ACID AND OTHER ACIDS[1,2]

ESMOND R. LONG AND FLORENCE B. SEIBERT

In the preceding article (study 1) the preparation of an active tuberculin from a nonprotein medium was described. After filtration from tubercle bacilli, first upon a Büchner funnel without paper, then through paper, and finally through a Mandler filter, a yellowish-brown but perfectly clear liquid was obtained, which had the characteristic odor of tuberculin, on concentration gave the common color tests for protein, and, in sharp contrast to the original medium, gave a flocculent precipitate with acetic acid.

In testing for the active principle of tuberculin in this preparation and products derived from it, the presence of the principle was detected by injecting appropriate amounts into the skin or testis of tuberculous guinea pigs, with injections into normal animals for control. Usually three tuberculous and two normal guinea pigs were used in a single test. A positive reaction in the tuberculous guinea pigs was taken as evidence of the presence of the active principle, provided negative reactions in the controls ruled out the possibility of primary toxicity in the product.

In the effort to determine the relation of the protein found in our tuberculin to the active substance in the fluid, that is, the substance responsible for the tuberculin reaction, an attempt was first made to precipitate the protein completely by a method which has proved of general application in the precipitation of colloids, namely, precipitation at its isoelectric point. Proteins are amphoteric, but most of them are more acidic than basic, that is, dissociate more H than OH ions. According to the law of mass action, increasing the concentration of H ions in the surrounding medium or solvent lessens the number of H ions dissociated by the dissolved protein. At a certain H-ion concentration of the solvent, the H and OH dissociations of the protein are equal, and the protein will

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

migrate neither to the cathode or anode in an electric field. This is the isoelectric point. At this point the solubility of the protein is at its minimum.

With the coöperation of Dr. I. S. Falk the reaction point of maximum precipitation and the isoelectric point were determined. The point of maximum precipitation was found by adding acetic acid to tuberculin in a series of test-tubes, and reading the reaction by the appropriate color indicator at the point where greatest precipitation occurred. Precipitation began in the neighborhood of pH 5.0, was greatest at 4.0, was decidedly less at 3.0 and almost vanished at 2.0. Identical results were secured with phosphoric acid. The amounts of acid it was found necessary to add to 5 cc. tuberculin and the amounts of precipitate produced at the various reactions were as follows:

	pH 5.0	pH 4.0	pH 3.0	pH 2.0
Cc. 10 per cent acetic acid	0.04	0.05	0.5	3.5
Precipitate with acetic acid	trace	++++	++	+
Cc. normal phosphoric acid	0.1	0.55	2.0	4.0
Precipitate with phosphoric acid	trace	++++	++	+

In spite of the established greater precipitating efficiency of the phosphoric acid with its trivalent ion, precipitation with phosphoric acid was not better than that obtained with acetic acid. The isoelectric point of the precipitate was found by Dr. Falk with the electrophoretic method to be pH 4.0. This figure agrees well with that of 4.2 obtained by Gabbe (1) for an active substance prepared from tubercle bacilli and from Old Tuberculin.

Tests of tuberculin activity, made by dissolving the acetic-acid precipitate in weak sodium hydroxide, neutralizing, and injecting intracutaneously into tuberculous guinea pigs, revealed high potency in the precipitated material. The precipitation and collection of the precipitate were so easy that this at first seemed the ideal method of obtaining the active principle in concentrated if not in pure form. Since nothing whatsoever precipitates on addition of acetic acid to the original medium, it may be assumed that everything which comes down on adding acetic acid to the same medium after growth of the tubercle bacillus is derived from the latter.

An account of the first collection made by this method illustrates its

convenience. Sixty-five litres of tuberculin were prepared by the Mulford Co. in the way described above, and the reaction at which maximum precipitation occurred was determined by trial in a series of test-tubes to which increasing amounts of acetic acid were added. Two hours were allowed for settling of the precipitate. An amount of acid corresponding to the concentration in the tube in which precipitation was maximum was then added to the whole. Seven hundred and fifty cubic centimetres of glacial acetic acid were required. The precipitate came down in large floccules. Precipitation was carried out in 10-litre bottles, which were allowed to stand over night for settling.

The supernatant liquid was siphoned off in the morning as completely as possible, and the last portions were removed by centrifugation in 500-cc. bottles. The flocculent precipitate was then suspended in 95 per cent alcohol and the mixture centrifugated. The process was repeated, the precipitate coming out as a clean, white mass. Finally the alcohol was removed by shaking the centrifugated mass in ether and centrifugating again. After pouring off the ether the precipitate dried rapidly to a white powder. Ten grams of product was prepared in this way from 65 litres of tuberculin.

The substance so obtained was of protein nature, giving the biuret, Millon, vanillin and glyoxylic acid tests. It was insoluble in water, but easily soluble in weak alkali. On neutralizing the alkaline solution and boiling, some coagulation occurred. The preparation contained 10.85 per cent water. On a water-free basis it contained 8.92 per cent nitrogen and 1.35 per cent phosphorus. The ash was surprisingly high, 7.74 per cent. A portion was hydrolyzed and xanthine found, but neither of the amino purines, guanine and adenine, could be detected.

The solution obtained by dissolving the product in weak alkali and neutralizing elicited a strong tuberculin reaction in tuberculous guinea pigs, and was quite nontoxic for normal animals. Skin reactions were provoked by 0.1 mgm. and 0.01 mgm., but not with 0.001 mgm. Testicle or spermatocyte reactions (2) (3) were set up by 0.1, 0.01 and 0.001 mgm.

However, it was soon apparent that precipitation was far from complete by this method. Even after maximum precipitation the filtrate from the precipitate, on neutralization of the added acid, invariably elicited a strong tuberculin reaction in a tuberculous guinea pig. It also gave the biuret, Millon, vanillin and glyoxylic acid tests for protein. On neutralization of the filtrate with calcium carbonate, filtration of precipitated calcium acetate and phosphate, and concentration *in vacuo*,

protein was precipitated by half-saturation with ammonium sulphate, and by addition of tannic acid, phosphotungstic acid and acetic acid.

Alcohol precipitation at the supposed isoelectric point also proved ineffective in causing complete precipitation. At the 1924 meeting of the National Tuberculosis Association, Long (4) reported the production of a highly potent substance prepared by combined acetic acid and 75 per cent alcohol precipitation of tuberculin prepared from a nonprotein medium. This combination of precipitants produced a heavy flocculation of organic material, but left most of the salts of the medium in solution. Later investigation proved that by this method, also, precipitation of the active principle was incomplete. The filtrate from the precipitated substance, on evaporation of the alcohol and solution of the residue in water, gave a strong tuberculin reaction in tuberculous animals. In the preliminary report of this alcohol-acetic acid precipitate it was stated that the precipitated substance did not give protein reactions. This was an error, probably due to the small quantities of material available at that time. Further study on larger quantities showed the material to be protein.

Two possibilities present themselves. One protein only may be present in our tuberculin, which precipitates poorly at its isoelectric point, or the tuberculin may contain several proteins of different isoelectric points. We have since obtained evidence for the presence of more than one protein giving the tuberculin test, as described in another article of this series. The isoelectric point, pH 4.0, determined by Dr. Falk, was probably the isoelectric point and point of maximum precipitation of one of these. At this point, however, other protein substances, no doubt closely related, remained incompletely precipitated. Had these been completely precipitated at their isoelectric point some of the protein precipitated at pH 4.0 would presumably have remained in solution. According to this hypothesis the filtrate from any single isoelectric-point precipitation would still contain some protein, and might give a tuberculin reaction. If several proteins or several modification products of one product are present, only by a fractional method of acid precipitation, with removal of each fraction before the next was precipitated, could one expect to throw out all of the protein present by this method.

We have since learned other methods, by which all of the protein and likewise all of the active principle can be precipitated, which are less laborious, and in the end probably more satisfactory, than a method of

fractional acid precipitation would be. These are reported in a later article of this series. Acid precipitation was therefore abandoned in our effort to identify active substances in our fluid tuberculin. The substance coming out at the maximum precipitation point was without doubt a mixture. The low nitrogen indicates that it was not all protein. The high phosphorus may be due to the presence of nucleoprotein. However, as Johnson and Brown (5) have shown, proteins which are not nucleoprotein but contain a high percentage of phosphorus are present in the tubercle bacillus.

The presence of xanthine in the hydrolyzed sample does not indicate the presence of nucleoprotein in the precipitated substance. Xanthine is derived from guanine by oxidation, following the breakdown of nucleoprotein. Intact nucleic acid contains guanine and adenine, but no xanthine or hypoxanthine. In the hydrolyzed product of our material, guanine and adenine were not present in detectable amounts. We may therefore feel certain that no appreciable amount of nucleic acid or nucleoprotein was present. Nucleoprotein is, however, present in the bacillus, and the presence of xanthine in our precipitate is probably to be attributed to autolysis of nucleic acid within the bacilli in the course of growth and metabolism, followed by diffusion into the medium. Undoubtedly, hypoxanthine was also present in the fluid tuberculin, but xanthine is the least soluble of the nuclear purines, and would be likely to come down in measurable amount adsorbed to the protein precipitate produced by acetic acid. It would be found unchanged in the hydrolyzed product of this precipitate.

Other acids proved no better precipitants than acetic. Hydrochloric acid, like acetic and phosphoric acids, threw down a protein which elicited the tuberculin reaction. In this case also the filtrate from maximum precipitation contained the active principle in amount sufficient to elicit strong skin tests in tuberculous guinea pigs.

Attempt was next made to precipitate the active principle by other acids as an insoluble salt with the negative ion. Tannic acid seemed the most likely to give good results. This acid was found by Koch (6) to precipitate the active principle, and Zinsser and Mueller (7) and others have used it with some satisfaction. However, we found it impracticable, because of the difficulty in separating the active principle from the tannic acid after precipitation of the tannate. The customary method is to use barium hydrate, but in our experience the tuberculin principle was lost in the voluminous barium tannate, perhaps by adsorption.

Trichloracetic and phosphotungstic acids have possibilites which are still under examination. Our results with these protein precipitants will be reported later.

Precipitation as a metal salt was also tried without success. The results of Mills (8), in the precipitation of proteins by mercury, gave much promise, but on trial it proved impossible to secure a good precipitate with mercuric compounds.

SUMMARY

From tuberculin made from a nonprotein medium acetic acid precipitates protein which elicits the tuberculin test in tuberculous guinea pigs. The point of maximum precipitation, obtained by adding acetic acid in increasing amount to tuberculin in a series of tubes, coincides with the isoelectric point found for a protein present, determined by electrophoresis. This point is pH 4.0. However, it is not the point of complete precipitation of all protein present. Protein remains in solution after maximum precipitation by acetic acid, and the filtrate from the precipitate still elicits a strong tuberculin reaction in tuberculous guinea pigs. It seems probable that a substance of protein nature is responsible for the tuberculin reaction, but that more than one protein is present in the tuberculin prepared from nonprotein media, and possible that more than one protein present can elicit a tuberculin test.

Phosphoric and hydrochloric acids behaved like acetic acid in the precipitation of this active protein. Tannic acid proved unsuitable for precipitation of the protein present, because of difficulty in removing the voluminous tannate from the precipitate. The mercuric ion was found entirely unsuitable in precipitating proteins present.

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THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

III. EXPERIMENTS ON DIALYSIS[1,2]

FLORENCE B. SEIBERT AND ESMOND R. LONG

Considerable disagreement exists in the literature concerning the ability of tuberculin to dialyze. A clear understanding of this property is important, especially when an attempt is made to purify or isolate the active principle from the tuberculin. Otherwise great losses may occur.

This property has been studied by many investigators, among whom are Zieler (1), Danielopolu (2), Löwenstein and Pick (3), and Zinsser (4), who claim that tuberculin, untreated or precipitated and redissolved, passes through dialyzing membranes of viscose, collodion, vegetable parchment, or fish-bladder. On the other hand, Marie and Tiffeneau (5), and Selter and Tancré (6) report that it is nondialyzable. Ruppel (7) states that tuberculin *A. F.* is not diffusible until it is subjected to heat, after which some of it passes through a membrane. Therefore, for our own satisfaction it has seemed worth while to determine whether or not our tuberculin preparations would dialyze.

Method: This study was greatly enhanced by the use of an automatic dialyzing apparatus, designed by Hanke (8), in which, by means of vacuum distillation at low temperature, the dialyzing sac rests continually in distilled water, and the entire diffusate, a maximum volume of 400 cc., can be preserved without loss for tests. In this way it is possible to detect the smallest trace of activity in the diffusate.

Fish-bladder membranes were used, and, since care in selection of the dialyzing membrane is of prime importance, only such bladders were used which, when filled with water and placed under pressure by means of twisting the sac, showed no oozing of water. A membrane should never be used twice, since there is evidence to indicate that repeated use renders it more permeable. This fact, together with the individual variations in permeability of membranes, may explain some of the varying results reported in the literature.

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

TABLE 1

SUBSTANCE TESTED	TIME OF DIALYSIS	VOLUME IN CUBIC CENTIMETRES AFTER DIALYSIS	VOLUME IN CUBIC CENTIMETRES AFTER CONCENTRATION IN VACUO	DOSE USED	SKIN TESTS		PROTEIN TESTS						
					Tuberculous guinea pigs.	Normals	Biuret	Millons	Vanillin	Glyoxylic	Ninhydrin	Precipitation with ammonium sulphate	Molisch
Experiment 1: 125 cc. dialyzed against 400 cc. (no preservative)													
				cc.									
Tuberculin (raw)				0.1	2+, 2+, 3+	−, −, −	−		+	+	+	+	
Nondialyzable residue	33 hours	240		0.1	1+, 1+, 3+	−, −, −	+	−	−	−	F	+	
Diffusate	33 hours	250		0.1	−, −, −	−, −, −	−	−	+	F	+	−	
Experiment 2: 125 cc. dialyzed against 400 cc. (chloroform preservative)													
Tuberculin (autoclaved)				0.1	3+, 3+, 4+	−, −, −	+	F	+	F	+	+	+
Nondialyzable residue	43 hours	250		0.1	2+, 3+, 4+	−, tr, tr	+ (P)	−	F	−	+ (R)	+	+
Diffusate	43 hours	250	150	0.1	−, −, tr 1+, 1+, 2+	−, −, −	−	−	+	F	+	−	+
Experiment 3: 100 cc. dialyzed against 400 cc.													
Tuberculin (raw)				0.1	* * *	−, −, −							
				0.03	5+, 5+, 5+	−, −	+		+	+	+	+	+
Nondialyzable residue	46 hours	337	237	0.1	5+, 5+, 5+	−, −, −	+		+	+	+	+	+
Diffusate	46 hours	125	125	0.1	−, 1+, 1+	−, −, −	−		+	+	+	F	+
Experiment 4: 90 cc. dialyzed against 400 cc.													
Tuberculin (raw)				0.1	6+, 6+	−, −	+		++	+	+	+	+++
Nondialyzable residue	5 days	230	54	0.1	5+, 6+, 6+	−, tr	+		++	+	F	+	+++
Diffusate	5 days	175	45	0.1	tr, tr, 1+	−, tr	−		++	+++	+	F	+++

F = faint reaction. P = pink color. R = red color. * = died.

From 75 to 125 cc. of tuberculin made upon synthetic medium (9), were subjected to dialysis in a fish-bladder membrane. Continuous dialysis against 400 cc. of distilled water for 33 hours to five days allowed practically none of the active principle to pass through the membrane. The diffusate then was tested directly, or concentrated *in vacuo* at 50° to 60°C. to a small volume, as noted in table 1.

Discussion of results: Four experiments were performed, as described above, and the complete results are tabulated in the accompanying chart. The skin reactions produced by injecting guinea pigs intracutaneously are recorded in table 1 in the columns designated *tuberculous guinea pigs* and *normals*. Negative reactions are indicated by −, trace by tr., and the definitely positive reactions range from 1+, a very mild reaction, up to 5+, an extremely severe reaction. The variations in reaction often noted are probably explainable by a difference in sensitivity of different animals, but since three guinea pigs were tested with each product a fair idea of the potency of the product could be obtained. The controls usually gave negative reactions, but occasionally one gave a trace of an inflammatory reaction.

In experiment 3 it is noteworthy that three guinea pigs were killed within 36 hours following an intracutaneous test with the raw tuberculin, and even 0.03 cc. gave a very marked reaction. This is the only one of many samples of tuberculin tested which was potent enough to cause death in this manner. Unusually rapid absorption must have occurred.

At a glance it can be seen in the table that almost none of the specific toxic material in tuberculin dialyzes, even when the dialysis is carried out for as long as five days (experiment 4). Furthermore, it is true that practically all of the protein remains in the sac during dialysis, as shown by the negative or faint precipitates obtained in the diffusate with saturated ammonium sulphate. The biuret test in the diffusate is also negative in all cases, but this cannot be used as an argument for the absence of protein to the same extent as the failure to precipitate with saturated ammonium sulphate, since glycerol has been shown to interfere with the biuret reaction (10).

In experiment 2 the same fish-bladder membrane was used as had been employed in experiment 1. In this experiment, also, the tuberculin used for testing had been previously autoclaved, and very little, if any more, active substance was found to dialyze than in the case with the raw tuberculin, contrary to Ruppel's findings. Results on dialysis of

protein fractions isolated from tuberculin will be included in a later paper in this series.

Parchment membrane was used in experiment 3, and results were obtained similar to those with fish-bladder membranes.

In experiments 2 and 4 all of the glycerol and practically all of the inorganic material dialyzed, as was demonstrated by the acrolein and ash tests. In every case, practically all of the substance responsible for the odor dialyzed. The diffusate also contained most of the color, which, however, was a greenish-yellow, in contrast to a brownish-yellow color remaining within the sac. This brownish-yellow color always seemed to be closely associated with the most potent fractions later obtained from tuberculin.

SUMMARY

1. Very little, if any, of the skin-reacting material of tuberculin, untreated or autoclaved, passes through dialyzing membranes (fish-bladders and parchment), even if allowed to dialyze as long as five days in a continuous vacuum dialyzing apparatus. This is true also for the protein in tuberculin. In this time, however, practically all of the inorganic material, odoriferous substance and glycerol dialyze.

2. A substance giving a positive Molisch test does dialyze to some extent, indicating that this carbohydrate is not especially associated with the skin-reacting material.

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THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

IV. AMMONIUM-SULPHATE PRECIPITATION OF THE PROTEINS OF TUBERCULIN[1, 2]

FLORENCE B. SEIBERT AND ESMOND R. LONG

Much effort has been expended in attempting to separate and identify the active substance in the culture media of tubercle bacilli, that elicits a skin reaction in tuberculous animals and not in normal animals. While many precipitants have been used from time to time (1), no serious effort has been made to determine what reagents will precipitate this substance completely from the mixture, and the majority of investigators have been content to precipitate only a portion of whatever is active for study.

By the nature of the problem, biological methods, namely, the skin and testicle tests, etc., have been the only means available for following the activity of various fractions, and these methods have been both laborious and somewhat unreliable. But it is only by the use of such crude tests as are at hand that we can hope to proceed to more accurate chemical methods of analysis.

In dealing with the tubercle bacilli themselves the problem of extracting from the solid mass active substances in unchanged form is at first a vital one. In dealing with the culture medium or crude tuberculin, however, the substances desired are already in solution, and methods for precipitating them are of prime importance. This paper will consider only products obtained from the tuberculin, and it will be of interest later to compare the products so obtained with those being isolated in the Yale laboratories from the very bacilli which have produced some of the tuberculin here used.

It is a striking fact that, throughout all of this work, whenever activity in a fraction was found, protein or protein derivatives were also present,

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

as indicated by the positive color reactions for proteins or their amino acids. And, on the other hand, whenever a fraction produced only weak or no skin reactions in tuberculous guinea pigs, that fraction usually gave no biuret reaction and only weak or no amino-acid tests. No very potent fraction has thus far been prepared which gave negative protein tests, although there have been a few cases in which mild potency was found when no protein could be detected. In most cases this apparent lack of parallelism in the tests can be accounted for. However, since the bulk of the evidence points to a close relationship between protein and the active substance of tuberculin, we naturally turn to the precipitants known to throw protein out of solution, in order to determine whether they also precipitate the active substance completely. Such reagents are alcohol, acetic and hydrochloric acids, tannic and picric acids, trichloracetic and phosphotungstic acids, potassium ferrocyanide, saturation with NaCl, Na_2SO_4, and $(NH_4)_2SO_4$, etc.

Koch (2) first separated the active substance from the medium with alcohol, and this has ever since been a popular method. Experiments performed in our laboratories indicate that alcohol is no better as a precipitant than are the acids mentioned, which have been treated in the second article of this series, and in respect to the bulkiness of the precipitate obtained, and the readiness with which it flocculates, this method is perhaps inferior. Experiments will be reported throughout this paper (see table 1) in which alcohol was used as the final means of obtaining a precipitate from a concentrated solution of the active substance, and in almost every case a large proportion of the activity remained unprecipitated. In still other experiments evidence is submitted to show that alcohol may even dissolve some of the active substance. Therefore, it would seem that alcohol precipitation is by no means a satisfactory method for removing the active principle from tuberculin or from extracts of the bacilli.

It has been found in this investigation that saturation with $(NH_4)_2SO_4$ precipitates from the tuberculin all of the active substance and at the same time all of the protein, and moreover, that the precipitate obtained with this salt is far bulkier and therefore easier to work with than those precipitates obtained by saturation with NaCl or Na_2SO_4. The $(NH_4)_2SO_4$ method, therefore, was adopted, and fractionation of the protein material according to standard methods was attempted. Moreover, products obtained in this manner can be compared with standard products reported in classical literature on the proteins. Koch (2),

TABLE 1

SUBSTANCE TESTED	SKIN TESTS			CHEMICAL TESTS						
	Dose	Normals	Tuberculous guinea pigs	Biuret	Millons	Vanillin	Glyoxylic	Ninhydrin	Heat coagulable	Molisch
Experiment A:										
One-half saturation solution	0.1 cc.	−, −	−, 1+	+		F	+	+	−	+
Complete saturation solution	0.1 cc.	−, −, −	2+, 4+, 4+	−		+	++	+	+	+
Filtrate	0.1 cc.	−, −, −	−, −, tr	−		F	F	−	−	+
Experiment C:										
(1) One-half saturation alcoholic precipitate	0.01 mgm.	−, −	4+, 5+	+	+	+	F	?	−	++
	0.001	−, −, −	tr, tr							
	0.0001	−, −	tr, tr							
	0.00001	−, −	−, −							
Alcoholic residue of (1)	0.1 cc.	−	−, −	−	−	+	−	−		+
(2) Complete saturation alcoholic precipitate	0.01 mgm.	−, −	2+, 5+	+	+	+	F	−	−	++
	0.001	−, −	1+, tr							
	0.0001	−, −	−, −							
Alcoholic residue of (2)	0.01 cc.	−	5+, 5+	−	−	+	−	F		++
(3) Filtrate	0.1 cc.	−, −, −	−, −, −	−	−	−	−	−		++
Experiment E:										
(1) One-half saturation alcoholic precipitate	0.1 mgm.	−, −	1+, 2+	+	+	+	F	−	--	−
Alcoholic residue of (1)	0.1 cc.	−, −	2+, tr	+	+	+++	+	R	−	++++
(2) Complete saturation alcoholic precipitate, water-soluble	0.1 mgm.	−, −	4+, 5+	+	+	F	−	−	−	+
Alcohol residue of (2)	0.1 cc.	−, −	3+, 5+	+	−	F	−	R	−	++

(3) Complete saturation precipitate later insoluble in water (heat-coagulable?)	0.1 mgm.	−, −	2+, 3+,4+	+	+	F	F	?	?	+
(4) Complete saturation water-insoluble alcoholic precipitate	0.1 mgm.	−, −	−, −, −	+	+	−	−	+	−	+
Alcohol residue of (4)	0.1 cc.	−, −	−, tr, 1+	−	−	F	−	−	−	++
Experiment B:										
Complete saturation alcoholic precipitate	0.1 mgm.	−	1+	+	−	−	−	−	−	+
	0.01	−, −	2+, tr							
	0.001	−	tr							
Alcoholic residue	0.1 cc.	−, −	2+, tr, −	−	−	F	−	−	−	+
Filtrate	0.1 cc.	−, −, −	−, −, −	−	−	−	−	−	−,	++
Experiment G:										
Complete saturation solution	0.1 cc.	−, −	3+, 5+	F	−	F	−	−	−	++
Filtrate	0.1 cc.	−, tr	−, tr, tr	−	−	−	−	−	−	++++
Experiment D:										
Three-quarter saturation alcoholic precipitate	0.1 mgm.	−, −	5+, 5+	+	+	F	+	+?	−	++
Alcoholic residue	0.1 cc.	−, −	tr, 2+	−	−	F	−	F		++
Filtrate	0.1 cc.	−, −, −	−, −, tr	−		++	+	−	−	++
Experiment F:										
Two-thirds saturation solution	0.1 cc.	−, tr	−, 1+	+	−	F	F	−	−	+
Filtrate	0.1 cc.	−, tr	2+, tr	−	−	−	−	−	−	++++

F = faint reaction. R = red color.

Marie and Tiffeneau (3), Malm (4), Lockemann (5), etc., have reported precipitation of the active substance by these salts, but none have emphasized the fact that these are so far the only means of precipitating the activity of the tuberculin completely, and that they are therefore superior to all other methods for analytical purposes. Dienes, Schoenheit and Scheff (6) state that precipitation with Na_2SO_4 removed all demonstrable traces of antigens from filtrates of acetic acid and heat precipitates.

EXPERIMENTAL

Table 1 shows that in six experiments (*A*, *C*, *B*, *E*, *F*, *G*) the dialyzed filtrate, after the precipitate obtained by complete saturation, or even three-fourth's saturation (*F*), of the tuberculin is filtered off, gives no skin reaction in tuberculous guinea pigs and also gives only faint or no protein and amino-acid tests. Therefore, all of the activity and all of the protein have been precipitated. Attention has been paid to the fact that the biuret test is masked by the presence of glycerol (7); therefore, unfair conclusions on this basis were guarded against. However, when the amino-acid tests are negative and when saturation with $(NH_4)_2SO_4$ gives no precipitate at the same time that the biuret test is negative, as in the case of most of these filtrates, it is safe to assume the absence of protein. Two-third's saturation with $(NH_4)_2SO_4$, as seen in experiment F, does not completely precipitate the active portion, and therefore three-fourth's saturation seems to be the lower limit for complete precipitation of the active substance.

Since saturation with $(NH_4)_2SO_4$ precipitates all of the active principle and all of the protein, it is of interest to know which protein fraction is accompanied by most of the activity; therefore, three experiments (table 1, *A*, *C*, and *E*) were conducted, in which proteins precipitated at half-saturation and at complete saturation with $(NH_4)_2SO_4$ were tested. Complete protocols of the preparation of the various fractions of 1/2, 2/3, 3/4 and complete saturation with $(NH_4)_2SO_4$ are given below, and the results (skin tests and chemical tests) are tabulated together in table 1 for the sake of comparison.

Protocol of Experiment A

Half-saturation fraction: 125 cc. of an untreated tuberculin, grown upon the nonprotein medium, was dialyzed in a fish-bladder membrane until it was odorless, free from salts, glycerol and free amino acids, and almost colorless. It

still gave a positive skin test. The volume had increased to 240 cc. To this was added an equal volume of a saturated solution of $(NH_4)_2SO_4$. A small amount of flocculent precipitate formed, which could not be centrifugated off because of the high specific gravity of the $(NH_4)_2SO_4$. Therefore, it was filtered through paper, and the precipitate dissolved in a small amount of n/10 NaOH. The alkaline filtrate was neutralized with HCl and then made to 50 cc., and dialyzed in a fish-bladder for 48 hours in the Hanke apparatus (8), after which time no precipitate was formed with $BaCl_2$, indicating the absence of SO_4.

Complete-saturation fraction: To the filtrate from the half-saturated $(NH_4)_2SO_4$ fraction, solid $(NH_4)_2SO_4$ was added to make complete saturation. The precipitate which formed was filtered off, dissolved in n/10 NaOH, neutralized, diluted to 50 cc., dialyzed and filtered through a Berkefeld candle, and tested in the same way as the former fraction. The final volume was 50 cc.

The filtrate from this second precipitate was dialyzed, concentrated to 50 cc., filtered through a Berkefeld candle, and then tested in guinea pigs.

Protocol of Experiment C

Half-saturation fraction: To 75 cc. of raw unheated tuberculin VIII, made upon the nonprotein medium, 75 cc. of a saturated solution of $(NH_4)_2SO_4$ were added. The flocculent precipitate was separated, and then dissolved in 15 cc. of distilled water, and dialyzed with toluol, for a preservative, in a fish-bladder for 60 hours. The resulting volume was 20 cc., which was concentrated *in vacuo* at 40°–50°C. to remove toluol and then filtered through a Berkefeld candle. It gave no precipitate with $BaCl_2$. To this filtrate were added nine volumes (or 180 cc.) of 95 per cent alcohol and the flocculent precipitate was centrifugated off, washed with alcohol and ether, and dried. The brown powder obtained weighed 9.6 mgm. For the skin and testicle tests this powder was dissolved in the smallest amount of n/10 NaOH possible, neutralized with HCl, and made to 9.6 cc., and from this solution further dilutions were made as indicated.

After the precipitation with alcohol, the alcoholic filtrate was evaporated to dryness, giving a cream powder. This was then taken up in alkali, neutralized with HCl and diluted to 10 cc., and 0.1 cc. portions were injected intracutaneously.

Complete-saturation fraction: After the precipitate was removed from the half-saturated $(NH_4)_2SO_4$ solution, to the filtrate of 150 cc. were added 53.2 gm. of solid $(NH_4)_2SO_4$, and another flocculent precipitate resulted. This was separated, and redissolved in 15 cc. distilled water, and dialyzed for 60 hours. The resulting volume of 20 cc. was treated from here on in the same

manner as the previous half-saturation fraction. The resulting brown powder weighed 6.5 gm.

The alcoholic filtrate was treated exactly as outlined above.

Filtrate from $(NH_4)_2SO_4$ precipitates: 75 cc. of the filtrate after complete saturation was dialyzed in a fish-bladder membrane until free from SO_4, as shown by the test with $BaCl_2$. The volume increased to 300 cc., and this then was concentrated *in vacuo* at 40°–50°C. to 75 cc., and then filtered through a Berkefeld candle. There was no color and no odor left and, as seen in the table, no protein or amino acids and no skin reaction were obtained.

Protocol of Experiment E

Half-saturation fraction: (1): Five litres of a tuberculin made at Parke, Davis & Co., by growing the tubercle bacilli for one month upon the nonprotein synthetic medium used before, were half-saturated with $(NH_4)_2SO_4$ by adding an equal volume of a saturated solution of $(NH_4)_2SO_4$. After standing over night, a brownish flocculent precipitate was filtered and centrifugated off, and then dissolved in about 200 cc. of distilled water, and dialyzed in parchment for six days with toluol. After this, no precipitate was obtained with $BaCl_2$. The toluol was removed *in vacuo* at 40°–50°C., and the clear brown solution was filtered through a Berkefeld candle. The filtrate (365 cc.) was concentrated *in vacuo* at 50°C. to 78 cc., filtered again through a Berkefeld candle, and tested in guinea pigs. To this solution nine volumes of alcohol were added and the precipitate centrifugated off, washed twice with 95 per cent alcohol and twice with ether, and then dried. The resulting brown powder weighed 227.6 mgm.

The alcoholic filtrate was filtered, concentrated *in vacuo* at 40°–50°C. to 15 cc., and finished to dryness in a vacuum desiccator. It was taken up in a minimum of alkali, neutralized with HCl, and made to 10 cc. for testing.

Complete-saturation fractions: (2): After the half-saturated fraction was removed, 3550 gm. of solid $(NH_4)_2SO_4$ were added to the 10 litres in order to completely saturate it. After standing two days the precipitate was filtered off through hardened filter paper. The precipitate was scraped off and suspended in distilled water until complete solution was obtained, filtered through paper (for residue see the next section), and the clear amber solution dialyzed in parchment with toluol for $6\frac{1}{2}$ days. Some SO_4 was still present, as indicated by a precipitate with $BaCl_2$. So the solution was dialyzed for two more days in a fish-bladder until is was free from SO_4. The toluol then was removed *in vacuo* at 40°–50°C., and the whole concentrated to 85 cc. A dark gummy precipitate, insoluble in water, was centrifugated off at this point, washed with alcohol and ether, and dried. It weighed 17.7 mgm.

(3): The supernatant solution from this insoluble precipitate was then

filtered through a Berkefeld candle, and this solution tested in guinea pigs. To the 80 cc. of filtrate resulting, nine volumes of 95 per cent alcohol were added and the precipitate was centrifugated off, washed several times with alcohol and ether, and dried. The resulting white powder weighed 58.2 mgm.

The alcoholic filtrate and washings were filtered and concentrated *in vacuo* at 50°C. to about 10 cc. and finished in a desiccator. This residue, when dry, was dissolved in a small amount of alkali, neutralized with HCl and made up to 10 cc. for skin tests.

(*4*): The fraction of the complete saturation precipitate which was insoluble in water, was now dissolved in n/10 NaOH, giving a deep yellow solution, and filtered through paper. It was neutralized with HCl and dialyzed for 6 days in parchment with toluol. It was concentrated *in vacuo* to 70 cc., filtered through a Berkefeld candle, and then nine volumes of alcohol were added. The precipitate obtained was dried with alcohol and ether as previously described. The weight of the dark gray powder was 29.8 mgm.

The alcoholic filtrate and washings were dried and tested in the usual manner.

Protocol of Experiment B

To 536 cc. of a dialyzed tuberculin made from the nonprotein medium were added 171 gm. $(NH_4)_2SO_4$, making 45 per cent saturation. A milky colloidal solution resulted. The whole was then concentrated *in vacuo* at 40°C. until solid $(NH_4)_2SO_4$ crystallized out, leaving a saturated solution. This was then filtered through paper, and the precipitate taken up in a small amount of alkali, neutralized with HCl, then dialyzed free from the NaCl in parchment for 60 hours, reconcentrated to 192 cc. *in vacuo*, filtered through a Berkefeld, and tested. An alcoholic precipitate of this solution was made, and the alcoholic filtrate taken to dryness, as described in previous protocols. Tests on these products are recorded in the table.

The $(NH_4)_2SO_4$ filtrate was dialyzed in parchment for 8½ days until it was free from $(NH_4)_2SO_4$, then concentrated *in vacuo* at 40°–50°C. to 122 cc., filtered through a Berkefeld, and tested.

Protocol of Experiment G

Fifty cubic centimetres of tuberculin made upon the nonprotein medium were saturated with solid $(NH_4)_2SO_4$. The precipitate was filtered off, dissolved in water, dialyzed free from $(NH_4)_2SO_4$ in a fish-bladder, concentrated *in vacuo* at 40°–50°C. to 40 cc., filtered through a Berkefeld candle, and tested. The filtrate was similarly treated and concentrated to 20 cc. and tested. The results are recorded in table 1.

Protocol of Experiment D

To 100 cc. of tuberculin made upon the nonprotein medium were added 300 cc. of a saturated solution of $(NH_4)_2SO_4$, making three-fourths saturation. The precipitate was filtered off, dissolved in water, dialyzed with toluol in a fish-bladder for five days, concentrated *in vacuo* at 40°–50°C. to a volume of 42 cc., and filtered through a Berkefeld candle. Then an alcoholic precipitate was made in the manner described previously, and tested. The alcoholic filtrate was also concentrated and tested. The $(NH_4)_2SO_4$ filtrate was dialyzed, concentrated to 80 cc., filtered, and tested also as usual. The results are contained in table 1.

Protocol of Experiment F

To 100 cc. of tuberculin made upon a nonprotein medium, 200 cc. of a saturated solution of $(NH_4)_2SO_4$ were added, making two-thirds saturation. The precipitate was filtered off, dissolved in alkali, neutralized with HCl, dialyzed for eight days in a fish-bladder with toluol, concentrated *in vacuo* to 20 cc., filtered through a Berkefeld candle, made to 50 cc. volume, and tested.

The filtrate was treated in the same way and concentrated to a volume of 50 cc., and tested.

Discussion of Experiments A, B, C, D, E, F, and G

In all six cases most of the activity was embodied in the complete-saturation fraction, while always the half-saturation fraction also gave some reaction. In experiment A it is easy to note this marked difference in potency in the two fractions. In experiment C the reaction obtained by 0.01 mgm. of the half-saturated fraction is to be compared with the reactions obtained by 0.01 mgm. of the alcoholic precipitate of the complete-saturation fraction plus that obtained with the alcoholic residue after this precipitation. This experiment illustrates, as do also *E*, *B*, and *D*, the inadequacy of alcohol as a precipitant and the fallibility of drawing conclusions based upon reactions obtained with the alcoholic precipitates alone. The same is true for experiment E, in which the skin reaction obtained with the half-saturated fraction is less than that obtained by the complete-saturation fractions, which include three fractions, namely (*2*) the alcoholic residue, (*3*) the fraction which at first was soluble in water and later became insoluble in water, possibly due to heat coagulation, and (*4*) the fraction originally insoluble in water plus its alcoholic residue. This last fraction (*4*) appears to be inactive and warrants more consideration (see later).

Therefore, based upon these experiments, the following plan was formulated: That, since the activity of tuberculin can be satisfactorily precipitated completely only by three-fourths to complete saturation with $(NH_4)_2SO_4$, and since wherever activity is found, there also is found protein, and, since the activity accompanies in various degrees of intensity the different protein fractions, an effort should be made to fractionate further the protein precipitate, with an idea of determining which fraction or fractions are the active substance or are closely adherent to the active substance; and, furthermore, of determining to what stage of protein evolution or decomposition this fraction belongs. In experiments *I*, *H*, and *K* an attempt has been made to carry out this plan.

Protocol of Experiment I

Twenty-five litres of tuberculin made by Parke, Davis & Co. upon the non-protein medium were saturated with solid $(NH_4)_2SO_4$. The bacilli had been planted upon 45 litres of medium 50 days previous to the time of filtration, and in this time 17 litres had evaporated. After the tuberculin and $(NH_4)_2SO_4$ were thoroughly mixed they were allowed to stand for a day and a half. A brown flocculent precipitate appeared. Because of the high specific gravity of the $(NH_4)_2SO_4$ it was impossible to remove the precipitate by centrifugation, even in a Sharpless centrifuge. Filtration through hardened paper was therefore attempted, but this method was so laborious and time consuming that finally filtration through a specially purified and washed asbestos was resorted to. The precipitate was then dissolved off of the asbestos in about 3 litres of distilled water, and dialyzed for 11 days with toluol in parchment sacs. About half of the solution was then concentrated to a volume of 50 cc. *in vacuo* at 50°C., and finished to dryness in a vacuum desiccator. This constituted a mixture of all the water-soluble fractions (*1*). The brown powder weighed 6.8 gm.

The other half of the solution was heated on a steam-bath for an hour in order to precipitate the coagulable protein, and then with very great difficulty this coagulated material was filtered off, but it was practically impossible to wash this material thoroughly because of its colloidal nature. Acid, autoclaving, etc., did not break the colloidal nature of the suspension, and it was therefore taken down to dryness on the steam-bath. The dark horny mass was then ground in a mortar and soaked in water over night. After this it was washed with water, alcohol and ether by centrifugation each time. The dry dark brown powder weighed 2.3 gm., (*2*), a water-soluble, heat-coagulable protein.

The filtrate from the coagulated protein was filtered through a Berkefeld candle to a clear amber solution, and then concentrated *in vacuo* at 50°C. to

TABLE 2

SUBSTANCE TESTED	SKIN TESTS			CHEMICAL TESTS							
	Dose	Normals	Tuberculous guinea pigs	Biuret	Millons	Vanillin	Glyoxylic	Ninhydrin	Diazo	Molisch	Heat-coagulable
Experiment I:											
(1) Mixture of water-soluble fractions...	0.1 mgm.	−, −	3+, tr, tr, −	+	++	++++	+++	+	+	?	+
(2) Heat-coagulable water-soluble.....	0.1 mgm.	−, −	2+, 4+, 5+, 5+	+	+	+	F	−	−	−	+
(3) Water-soluble noncoagulable.....	0.1 mgm.	−, −	2+, 3+, 5+	+(P)	F	+	F	+	+	++++	+−
(4) Water-insoluble (combined with same from *H*).....	0.1 mgm.	−, −	1+, 1+, −	+	+	F	F	(R)	−	−	−
(4a) Water-insoluble.....	0.1 mgm.	−, −	1+, 1+, 3+	+	−	+				+	
(5) Filtrate.....	0.1 cc.	tr, tr	tr, tr, tr	?		++(O)	+(B)	−		+++	−

F = faint reaction. R = red color. O = orange color. B = blue color.

about 50 cc., and finished to dryness in a vacuum desiccator. The weight of the dry, medium-brown powder was 2.8 gm., (*3*), a water-soluble, noncoagulable protein.

A black gelatinous precipitate stuck to the asbestos or cloth used for filtering off the water-soluble fractions, even after considerable washing with water. It was insoluble in water, but soluble in n/0.1 NaOH, giving a deep brown, clear solution. It was then neutralized with HCl, and a faint cloudiness appeared. After dialysis for ten days in parchment with toluol, it was concentrated *in vacuo* to a small volume, and finished in a vacuum desiccator. Because of an accidental decrease in water pressure this fraction and approximately an equal amount of the comparable fraction from experiment H were precipitated by desiccator H_2SO_4. The precipitate was washed by centrifugation with water four times until the supernatant water was no longer acid to litmus, then with alcohol three times and ether once and dried. The resulting cream-colored powder weighed 1.8 gm., (*4*), a water-insoluble protein.

An insoluble fraction, (*4a*), obtained in a similar way from 8 litres of tuberculin without precipitation with H_2SO_4 is recorded in (*4a*).

A sample of the filtrate (250 cc.) from the $(NH_4)_2SO_4$ precipitation was dialyzed free from SO_4 for 11 days, concentrated to 50 cc. *in vacuo*, filtered through the Berkefeld candle, and tested (*5*).

The results of chemical and skin tests are tabulated in table 2.

Discussion of Experiment I

From the experimental data obtained, it is apparent that the two water-soluble fractions, as prepared from the whole $(NH_4)_2SO_4$ precipitate, that is, the heat-coagulable and noncoagulable, are the most potent of all fractions obtained. The mixture of the water-soluble fractions was less potent than either of the two separate fractions obtained from it. Moreover, the water insoluble fractions contain very little of the active substance.

This experiment supports previous work in the fact that after filtering off the precipitate obtained by complete saturation with $(NH_4)_2SO_4$ no activity is left in the filtrate.

Protocol of Experiment H

(*1*): Thirty litres of tuberculin made at the Mulford Co. on the synthetic medium were three-quarters saturated with $(NH_4)_2SO_4$ by addition of three volumes of a saturated $(NH_4)_2SO_4$ solution, and then filtered by gravity through large hardened filter papers. The precipitate was scraped off the papers and suspended in water, and the papers thoroughly washed with water. The

washings and precipitate were mixed and, because of the quantity of $(NH_4)_2SO_4$ crystals, a saturated solution of $(NH_4)_2SO_4$ resulted. The precipitate which formed was filtered off through hardened paper and resuspended in about 300 cc. of distilled water, dialyzed free of SO_4 in parchment with toluol for nine days, filtered through a Berkefeld, concentrated *in vacuo* at 40°–50°C. to about 100 cc., and then finished to dryness in a vacuum desiccator. The weight of the dry brown powder, which was a mixture of all the water-soluble fractions, was 15.2 gm. (*1*).

The toluol layer, which was used to preserve the protein during dialysis, became colored a deep orange, in contrast to the usual colorless toluol. It was therefore evaporated to dryness, leaving a heavy red fatty layer. This was taken up in alkali, and neutralized with acid. A milky suspension resulted, and it can be seen in table 3 that this fraction gave a very marked skin reaction and scarcely any protein reaction.

The diffusate was concentrated to a very small volume and tested in guinea pigs.

The fraction of the entire $(NH_4)_2SO_4$ precipitate, which was insoluble in water, was treated exactly like the comparable fraction in experiment I and in fact, as there mentioned, was mixed with it in the final stages. Therefore, for data refer to that experiment (table 2).

(*2*): The whole water-soluble fraction was later fractionated further. Fourteen grams were dissolved in distilled water, and a small portion remained insoluble (*2*). Since the whole was originally soluble in water, the present insolubility of a small fraction was probably due to heat coagulation. The precipitate was filtered off and washed thoroughly with water, and then dissolved in a small amount of alkali. Upon neutralization, a white flocculent precipitate came out, and this was filtered off with great difficulty. After filtration it appeared as a brown pasty precipitate. It was redissolved in NaOH, filtered and neutralized with HCl, and again a white flocculent precipitate resulted. This was filtered off, washed twice with water, once with 95 per cent alcohol, twice with ether and dried. A fine white powder (0.2 gm.) resulted, (*2*), a water-soluble, heat-coagulable protein.

(*3*): The filtrate from this insoluble fraction was a clear dark brown viscous solution. It was heated to 80°–90°C. for one hour, and then the coagulated protein filtered off with great difficulty through hardened paper and then through a Berkefeld candle, requiring several days. This precipitate, which was pinkish white, stood in water for three days, and then was filtered off and washed with distilled water many times until the wash-water was a colorless clear solution. The coagulated precipitate was then washed with 95 per cent alcohol and ether twice, and dried. The cream-colored powder weighed 2.3 gm., (*3*), a water-soluble, heat-coagulable protein.

(*4*): The clear brown viscous filtrate, after the coagulated protein was

TABLE 3

SUBSTANCE TESTED	SKIN TESTS			CHEMICAL TESTS							
	Dose	Normals	Tuberculous guinea pigs	Biuret	Millons	Vanillin	Glyoxylic	Ninhydrin	Diazo	Molisch	Heat-coagulable
Experiment H:											
(1) Mixture of water-soluble fractions	0.1 mgm.	−, tr, −	5+, 5+, 5+	++	++	++++	+++	+++	+	++	+
	0.01		2+, 3+								
	0.001		tr, tr								
	0.0001		tr, −								
Toluol extract of (1)	0.1 cc.	−, tr	1+, 2+, 5+	−	?	?	F	−	F	−	
Diffusate of (1)	0.1 mgm.	−, −	3+, 3+, 5+	F	−	F	F	+++	−	+	−
(2) Heat-coagulable (?)	0.1 mgm.	−, −	3+, 5+	++	++	++		+	+++	+	−
(3) Heat-coagulable water-soluble	0.1 mgm.	−, −	2+, 4+	++	++	++++		F	+++	+	−
(4) Noncoagulable water-soluble	0.1 mgm.	−, −	4+, 5+, 5+	+	−	+		−	+++	++++	−
(5) Diffusate of (4)	0.1 cc.	−, −, tr	2+, 3+, 4+	?	−	?	+	−	F	++	−
		−	2+								

F = faint reaction.

removed, did not cloud when heated to boiling. The filtrate (300 cc.) was concentrated *in vacuo* at 50°–70°C. to 115 cc., and then saturated with 86 gm. recrystallized $(NH_4)_2SO_4$. A heavy, creamy precipitate of pasty consistence was filtered off, and washed with saturated $(NH_4)_2SO_4$ solution. It was redissolved in 90 cc. water, filtered through hardened paper, reprecipitated with 84 gm. recrystallized $(NH_4)_2SO_4$, and a milky, creamy precipitate resulted. This was filtered off through hardened paper, washed with saturated $(NH_4)_2SO_4$ solution and redissolved in 100 cc. water, and filtered again. A small amount of insoluble brown gummy residue was left on the paper at this filtration. The filtrate was then filtered with great difficulty through a Berkefeld candle to a clear brown solution (100 cc.). It gave a pink biuret test. It was dialyzed then in a fish-bladder in the Hanke dialyzing apparatus against 400 cc. of distilled water for 35 hours. It was then filtered through a Berkefeld candle and concentrated *in vacuo* at 40°–50°C, and finished in a vacuum desiccator to dryness. The dark brown powder weighed 4.0 gm., (*4*), a water-soluble, noncoagulable protein.

The dialysate, (*5*), of this noncoagulable water-soluble fraction contained all of the SO_4, and was greenish-yellow in color. It was concentrated *in vacuo* to 100 cc., and no attempt was made to free it from SO_4 or from the flocculent precipitate which appeared, since the presence of dialyzable proteoses and other protein derivatives was being sought. Therefore, it was injected as it was into guinea pigs. For the results obtained see table 3.

Discussion of Experiment H

From the data in table 3 it is seen that the whole unfractionated water-soluble fraction was only slightly more potent than either of the fractions separated from it. However, if consideration is paid to the amount of activity that may have been lost from any one fraction by dialysis, then we would name the fractions as follows, in order of intensity of reaction produced by them: Mixture of water-soluble fractions or the noncoagulable fraction almost equally potent, much less activity in the heat-coagulable fraction, and practically none in the water-insoluble fraction.

In view of the consistent results reported in study III of this series, in regard to the nondiffusible nature of the active principle of whole tuberculin, it is of considerable interest to note that some active substance does pass through the dialyzing membrane, when the noncoagulable water-soluble fraction, isolated from the tuberculin, is dialyzed. See, for example, the results with diffusates in table 3 (*1*) and (*5*). The same result was obtained in experiment K, and the significance and interpretation must wait for further experiments.

In this experiment, for the first time, the toluol used as preservative for the water-soluble fraction during dialysis gave a very marked skin reaction and was also colored a deep orange.

Protocol of Experiment K

Forty-nine litres of tuberculin made upon the synthetic nonprotein medium at Parke, Davis & Co. were saturated with solid $(NH_4)_2SO_4$, and the precipitate filtered off by gravity through large hardened filter papers. A portion of the filtrate (*4*) was dialyzed free from $(NH_4)_2SO_4$ in parchment with toluol for 14 days, then concentrated *in vacuo* at 40°–50°C. to about one-tenth of its original volume, and tested in guinea pigs. Another portion of the filtrate was not dialyzed, but simply concentrated *in vacuo*, filtered free from the solid crystals of $(NH_4)_2SO_4$, and tested in guinea pigs. In table 4 the reactions with this solution which contained $(NH_4)_2SO_4$ are marked as negative in both normal and tuberculous guinea pigs, because they were simply hyperemic areas, which in thirty-six hours turned into surface crusts, and there was no induration, as seen in a typical tuberculin skin reaction. In other words, the reaction produced was probably due to the $(NH_4)_2SO_4$ present, and was not a tuberculin reaction.

The $(NH_4)_2SO_4$ precipitate was scraped from the papers, dissolved in water, dialyzed with toluol for 14 days, and then filtered free from a water-insoluble mucilaginous material (*1*). This water-insoluble precipitate was washed with water with much difficulty, requiring several days, and the filtrate was still slightly yellow. The precipitate was dissolved in alkali to a clear brown solution, filtered, neutralized with HCl, and finally precipitated with HCl at the point of maximum precipitation. It was redissolved in NaOH, and reprecipitated with acid and centrifugated off. It was soluble in both dilute acid and alkali. On dehydrating with alcohol (95 per cent), a portion dissolved, as indicated by the color of the solution, and so this alcoholic extract was separately dried and tested. The precipitate itself was then treated with ether and dried, a water-insoluble protein.

The acid filtrates and water-washes from this insoluble fraction were collected, and tested in guinea pigs, and, as the table shows, they also contained both protein and activity.

The aqueous filtrate containing the water-soluble fractions was finally filtered clear through a Berkefeld candle, and then was further divided into several fractions. It was first concentrated *in vacuo* to about 700 cc., and then heated up to 70°C for several hours to permit coagulation. This coagulated protein was filtered off and washed with extreme difficulty, (*2*), water-soluble heat-coagulable fractions.

A part of the coagulated protein was dissolved to a clear brown solution in

10 per cent NaOH, filtered through paper, and neutralized with HCl. The precipitate was washed with water, alcohol and ether, and dried (*2a*).

The other portion of the coagulated protein was simply dehydrated with alcohol and ether, and dried (*2b*).

The alcohol used for dehydrating in both of these cases dissolved a small portion of the precipitate, and these extracts were tested separately in order to see whether the loss was very great. In the case of (*2a*) the extract was fatty and brown.

The clear brown filtrate, from the water-soluble noncoagulable fraction, (*3*), was concentrated to 400 cc., but no further because of foaming. It was a very dark brown solution, and upon concentration a brownish precipitate appeared. The whole suspension was tested in guinea pigs, (*3b*), a residue after removal of $(NH_4)_2SO_4$ precipitate.

This $(NH_4)_2SO_4$ precipitate of the noncoagulable fraction, which had been filtered off after saturation, was dissolved in the smallest amount of water possible, and filtered through paper and then through a Berkefeld candle to a clear solution. This 260 cc. was then concentrated *in vacuo* at 50°–60°C. to 125 cc., and then dialyzed in the Hanke apparatus against 400 cc. water for two days with toluol. The diffusate was concentrated to 75 cc., and a brown precipitate appeared. The entire suspension was tested in guinea pigs, (*3a*), a diffusate of the noncoagulable fraction.

The nondialyzable residue was free from $(NH_4)_2SO_4$. A chocolate precipitate, (*3c*), had appeared during the dialysis. This was filtered off with great difficulty, and may represent a heat-coagulable protein similar to (*2*). It was dissolved in alkali, and precipitated with acetic acid at maximum precipitation point, washed, dehydrated and dried. The acetic-acid filtrate from this precipitate was concentrated *in vacuo* and also tested in guinea pigs, (*3d*).

The solution remaining after the insoluble fraction appearing during dialysis was removed, was then filtered through a Berkefeld candle to a crystal clear solution, and concentrated *in vacuo* at 50°–60°C. to about 20 cc., and finished in a vacuum desiccator to dryness (*3*).

Discussion of Experiment K

The data in table 4 show that in this experiment a separation was not made between potent and nonpotent protein fractions, since the water-soluble as well as the water-insoluble fractions were active. The fact of most importance brought to light in this experiment is that after the water-soluble noncoagulable fraction had been precipitated with $(NH_4)_2SO_4$ and redissolved several times, the activity dialyzed, as in the former experiment.

The $(NH_4)_2SO_4$ filtrates, (*3b*) and (*4*), after complete saturation always

TABLE 4

SUBSTANCE TESTED	SKIN TESTS			CHEMICAL TESTS							
	Dose	Normals	Tuberculous guinea pigs	Biuret	Millons	Vanillin	Glyoxylic	Ninhydrin	Diazo	Molisch	Heat-coagulable
Experiment K:											
(1) Water-insoluble	0.1 mgm.	−, −?	2+, 5+	+	F	++	+	−	++	+	−
Alcoholic extract of (1)	0.1 mgm.	−, −	1+, 1+, tr.	−?	−	+	−	−		?	?
Filtrate of (1)	0.1 cc.	−, −	2+, 3+	+(P)	−	4+	+	+++	−	+	−
(2) a. Heat-coagulable, water-soluble (acid precipitate)	0.1 mgm.	−, −	3+, 4+	+(P)	++	+	+	F	−	+	−
Alcoholic extract of (2a)		−, −, −	−, 4+, tr, tr	+	+?	+	+	−	−	+	+
b. Heat-coagulable, water-soluble	0.1 mgm.	−, tr	2+, 3+	+	F	++	+	−	F	+	−
Alcoholic extract of (2b)		−, −?	tr, tr	−	−?	4+	−	4+	−	++	−
(3) Water-soluble noncoagulable	0.1 mgm.	−, −	3+, tr	+	−	++	+	−	F	+	−
a. Diffusate	0.1 cc.	tr, tr?	1+, 3+, 4+	+(P)	4+	4+	4+	4+	++	4+	−
		−, −	1+, 3+								
b. $(NH_4)_2SO_4$ filtrate	0.1 cc.	−, −	−, −	+(P)	4+	4+	++	4+	++	4+	+
c. Heat-coagulable (?) acid precipitate	0.1 mgm.	−, −	tr, 1+	+(P)	++	+	+	−	−	+	−
d. Filtrate from c	0.1 cc.	−, −	tr, 2+	+(P)	−	F	F	F	++	−	−
(4) Filtrate from whole precipitate:											
Dialyzed	0.1 cc.	−, −	−, −	−	−	?	−	+	−	4+	−
Undialyzed	0.1 cc.	−, −	−, −	−	−	?	F	4+	F	4+	−

F = faint reaction. P = pink color.

were inactive, regardless of whether they were dialyzed or not before testing, or whether the precipitate was the last of a series of precipitations.

Slight reactions were obtained with alcoholic extracts of some of the fractions, and these were extracts made of the precipitates during dehydration. Therefore, as mentioned before, not only is alcohol a poor precipitant for the active principle, but also in some cases it even dissolves a small amount of the active substance.

GENERAL DISCUSSION

The presence of protein as a constituent of tuberculin, even when it is made from a nonprotein medium, has been a constant finding throughout this investigation, and serves as an essential basis for continued study. Whether protein is excreted during the metabolism of the bacteria, or whether it is extracted from the bacilli during their growth or after death by the medium, are questions still open for investigation. But that protein in tangible amounts is definitely present after the tubercle bacilli have been allowed to grow in a medium, which at first contained no protein, is not to be questioned.

In attempting to isolate from tuberculin the substance which gives the tuberculin skin reaction, it was soon recognized that the only reagents which precipitated the activity could be grouped as true protein precipitants, notably $(NH_4)_2SO_4$ in full saturation. These are the reagents which have been used by all investigators for this purpose, but nowhere has it been emphasized that $(NH_4)_2SO_4$ is the only reagent which precipitates the activity completely and that other precipitants, such as alcohol, acetic acid, HCl, etc., precipitate only a part. In this work it has been repeatedly shown that when tuberculin is saturated completely with $(NH_4)_2SO_4$ and the precipitate filtered off, the dialyzed filtrate, even after considerable concentration, elicits no skin reaction in tuberculous guinea pigs, and this result was not obtained with any other reagent used for precipitation. Three-fourths saturation with $(NH_4)_2SO_4$ will practically completely remove the activity, but this seems to be the lower limit, since a filtrate after two-thirds saturation contained recognizable activity. The presence of activity is closely paralleled by the presence of protein as determined by the color reactions for protein groups. In these experiments, when the protein was completely removed from the tuberculin by means of the $(NH_4)_2SO_4$, as indicated by the protein tests, then always the activity was also completely removed. Dienes, Schoen-

heit and Scheff (6) also concluded "that there is a rough parallelism between the potency of preparations in the skin test and complement fixation, and between them and their content of coagulable protein."

In attempting to fractionate the activity along with fractionation of the protein it was found that half-saturation with $(NH_4)_2SO_4$ did not remove as active a fraction as did subsequent complete saturation with the same salt, although no clear-cut dividing line could be recognized. Therefore, other methods for isolating distinguishably different proteins were sought.

For example, it was evident that a separation could be effected upon the basis of solubility. Most of the precipitate produced by saturation with $(NH_4)_2SO_4$ was soluble in water, but a portion, which usually was very mucilaginous and associated with a brown, almost black pigment, was insoluble in water but went into solution in dilute NaOH. It was also not coagulable by heat, and is probably a protean. This fraction, on the whole, gave very little or no reaction, except in the last experiment, in which it was somewhat potent. Because of the mucilaginous colloidal nature of this water-insoluble precipitate, great difficulty was met in filtering it, and even greater difficulty in washing it with water in order to effect a clean separation of the two fractions. It is therefore still a possibility that any activity in this fraction may be due to adhering contamination, which was not washed out, and this explanation seems all the more plausible since the first precipitate studied, which was small in quantity compared with later ones, contained no activity.

From the portion of the $(NH_4)_2SO_4$ precipitate which went into solution in water, two different proteins could be further separated on the basis of coagulability with heat. In each case practically half of the water-soluble fraction was coagulable, and here, as before, the filtration of one from the other was extremely difficult. In all three experiments this fraction was about as potent as the noncoagulable fraction filtered from it, but here, as before, the great difficulty in filtering and washing of the heat-coagulable fraction may account for its activity, in so far as some of the noncoagulable fraction may not have been thoroughly washed away.

On the other hand, on the basis of Zinsser's work (9), in which he claims that it is practically impossible to remove coagulable protein completely from solution by boiling, it is conceivable, although improbable, that the activity of tuberculin is primarily a function of this coagulable protein, and that the noncoagulable fraction filtered from it reacts simply because

traces of the former protein resisted coagulation and went through with the filtrate.

The filtrate containing the noncoagulable water-soluble fraction always gives a very marked skin reaction, even after filtration through a Berkefeld candle to a clear brown solution. It is worthy of note that the brown color seems to be closely associated with this protein, since the aqueous solution is always colored a deep brown, and also since most of this color remains with the activity inside of the membrane during dialysis, and also since repeated saturation with $(NH_4)_2SO_4$ and filtration do not materially decrease the amount of color in the final product.

The potency of the water-soluble fraction was not increased through repeated purification by resaturating with $(NH_4)_2SO_4$ and filtering off the precipitate, and this may be explained in one of several ways. In the first place, it may be that the limit of tuberculin sensitivity has been reached and that a solution containing 0.001 gm. per cc. of dry tuberculin is as dilute as will be detected by means of a skin reaction of this type. Or, if the protein obtained is the active principle and if it is very highly purified, then further concentration of potency cannot be expected.

A second interpretation would be the possibility of a balancing of two simultaneous processes during purification, which would tend to prevent an increase in potency, namely, the production of the active substance from a nonactive material, and the destruction of this active principle to a nonactive substance.

The fact that activity does not pass through the membrane upon dialysis of untreated tuberculin (see study III), whereas a considerable amount does dialyze from the potent water-soluble noncoagulable fractions isolated from the tuberculin, is very significant. This dialysis of activity occurred on too many different occasions to be considered merely accidental or due to a leaky sac, and may indicate that an active dialyzable prosthetic group is gradually loosened from the protein molecule during continued treatment with $(NH_4)_2SO_4$ or mild heat. On the other hand, it may be interpreted to mean that some of the very first cleavage products of protein, namely, the large proteose molecules which will just dialyze, are still active and therefore responsible for the activity in the diffusates. This last interpretation is the most probable, and finds support in the data which show that these active diffusates usually give a pink biuret reaction.

Upon examination of the tables it is evident that there is no parallelism between the presence of skin-reacting material and a positive Molisch

test. On many occasions no skin reaction is given by a product which gives the most positive Molisch test, and vice versa. Therefore, the carbohydrate present in tuberculin is in no way connected with the skin-reacting material, and this supports Mueller's findings (10) in regard to the gum isolated by him from tuberculin.

SUMMARY

Protein is produced in considerable amount during the growth of tubercle bacilli upon a nonprotein medium.

The active principle of tuberculin and the tuberculoprotein are thrown out of solution by protein precipitants, and are precipitated completely only by three-fourths to complete saturation with $(NH_4)_2SO_4$. Two-thirds saturation just fails to precipitate it completely.

Although complete saturation with $(NH_4)_2SO_4$ precipitates a tuberculin fraction which is very potent, the fraction precipitated by half-saturation also contains considerable activity, and no clear-cut separation of the active principle can be made in this way.

The protein substance obtained by complete saturation of tuberculin with $(NH_4)_2SO_4$ has so far been fractionated into three proteins with definitely different properties:

(1) A water-insoluble, noncoagulable protein.
(2) A water-soluble, noncoagulable protein.
(3) A water-soluble, coagulable protein.

The water-insoluble fraction usually contains comparatively little activity. The two water-soluble fractions are about equally potent in most cases. While one can be reasonably sure in the case of the noncoagulable fraction that the activity is due to this fraction *per se*, the same cannot be equally held for the heat coagulable fraction, because of the great difficulty in filtering and thoroughly washing protein precipitates.

Although practically no activity dialyzes away from untreated tuberculin, considerable active substance does pass out of the membrane when the active water-soluble noncoagulable fraction isolated from the tuberculin is dialyzed. Whether this potent diffusible material is a loosened toxic prosthetic group, or whether it is an active proteose molecule hydrolyzed from the protein, are questions still open to investigation, although the latter argument would seem to have slightly more support.

This dialysis of activity may account to some extent for the failure to increase the potency of the water-soluble noncoagulable fraction by continued purification.

A record of the Molisch tests on these different fractions indicates that the carbohydrate material in tuberculin has no direct association with the skin-reacting material.

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THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

V. THE EFFECT OF PROTEOLYTIC ENZYMES ON TUBERCULIN PROTEINS AND THE ACTIVITY OF TUBERCULIN[1,2]

FLORENCE B. SEIBERT

The protein nature of the active principle of tuberculin has been disputed since Koch's time (1). However, the presence of protein in tuberculin, even when the latter is made by growing tubercle bacilli upon nonprotein medium, is now clearly recognized, but just what relationship this protein has to the substance to which tuberculous guinea pigs are sensitive has not been understood.

It was seen in study IV of this series that it is possible to remove all of the active substance or specific skin-reacting material from tuberculin simultaneously with all of the protein, but this fact alone does not prove that the two things are identical. As a matter of fact, all of our work so far has consistently indicated that the active principle of tuberculin is associated with protein or protein-decomposition products, and therefore any tests with the most specific protein reagents that we know, namely, the proteolytic enzymes, should afford valuable data. This is especially true, in view of a recent address by Hunter (2) in which he emphasizes this method of approach in the study of protein structure. This has long been recognized and many studies of the effect of the proteolytic enzymes upon the activity of tuberculin have been made.

Among the investigators have been Baldwin and Levene (3), who claimed that trypsin destroys the activity of tuberculin while pepsin merely weakens it, and that these facts suggest that the substance is of nucleoprotein nature. Pfeiffer (4) reported that trypsin and pepsin both destroy tuberculin activity while erepsin has no effect. Danielopolu (5), Franceschelli (6), Kinghorn (7), Carriere, Koehler, Persch, Loeffler (8), Löwenstein and Pick (9), Mueller (10) and others claim that pepsin as well as trypsin destroys the active substance.

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

EXPERIMENTAL

A few preliminary experiments for orientation were performed upon untreated tuberculin. It was found that if equal parts of the tuberculin and 0.02 per cent pancreatin, with or without 0.5 per cent Na_2CO_3, or 0.3 per cent pepsin in 0.2 per cent HCl were mixed and incubated for about nine days, practically all of the activity of the tuberculin had disappeared, as shown by negative skin tests in tuberculous guinea pigs. If the enzymes were destroyed by boiling before mixing, then there was no decrease in activity, while 0.5 per cent Na_2CO_3 did reduce it about half. If the tuberculin and pancreatin were tested immediately after mixing, no loss in activity was noted, indicating that it is not simply a neutralization effect.

Since these results supported the investigations of many previous workers, it seemed advisable to study quantitatively the effect of the various proteolytic enzymes upon a single, highly purified and very potent protein isolated from the tuberculin. The water-soluble, noncoagulable protein, described in the preceding paper, was chosen, since this fraction would be less likely to be contaminated by the other proteins and since it always gave a marked skin reaction. It will be known as tuberculin protein-X.

At first an attempt was made to follow simultaneously the loss in biological activity of the tuberculin protein-X, the change in pH, and the freeing of amino nitrogen, during digestion at 37°C. In a later series the actual breakdown of the whole protein molecule through proteose to amino acids and ammonia by enzymes was followed chemically, along with the loss in biological activity. This procedure, then, should enable us to determine with which fraction of protein or protein-decomposition product the activity is associated.

Method: Pawlow-pouch gastric juice was used as a source of pepsin, since there is no chance of its being contaminated with pancreatic juice and it is less likely to contain erepsin than the commercial preparations. (It is a pleasure to express my appreciation to Drs. A. Luckhardt and A. C. Ivy for supplying this juice.) Fairchild's trypsin was used. Erepsin was made in the usual manner from fresh dog intestinal mucosa.

A 10 per cent aqueous solution of tuberculin protein-X was used, and in each case 1 cc. of this solution (containing 0.1 gm.) was made to 10 cc., with either undiluted gastric juice, 9 cc. of a 0.1 per cent trypsin solution, 9 cc. of a 0.1 per cent trypsin containing 0.3 per cent Na_2CO_3,

9 cc. 10 per cent erepsin emulsion, 9 cc. distilled water, 9 cc. 0.3 per cent Na_2CO_3, 9 cc. 0.2 per cent HCl, or 9 cc. 0.46 per cent HCl. These mixtures were incubated under toluol up to ten days at 37°C. Total nitrogen was determined upon 1 cc. (containing 0.01 gm. protein) duplicate samples by the microkjeldahl method, titrating with N/0.01 acid and alkali with alizarin. Amino nitrogen was determined on 1-cc. samples by Van Slyke's method.

Whole protein and proteose were determined by Hahn's method (11). Since this method is not used in a widely routine manner, a brief description here of the technique will not be out of place. The whole protein from 2-cc. samples of this 1 per cent protein solution was precipitated by 4 cc. 20 per cent trichloracetic acid, and after standing for 10 to 15 minutes the precipitate was filtered off through hardened filter paper and thoroughly washed. The nitrogen in the entire filtrate was determined by the micro-method. The difference between half of this nitrogen and the total nitrogen in 1 cc. of solution gives the nitrogen in the precipitate or the nitrogen which represents the precipitated whole protein.

Similarly, the proteose nitrogen was determined on separate 2 cc. samples of the protein solution by precipitating them with 3 cc. 5 per cent phosphotungstic acid containing 3 per cent H_2SO_4. This precipitate always settled out very rapidly, and was likewise filtered off through hardened filter paper, washed thoroughly, and nitrogen run upon the entire filtrate. Half the amount of nitrogen in this filtrate, or the amount of amino and residual nitrogen not precipitated from 1 cc. of the protein solution, subtracted from the amount of nitrogen in the trichloracetic-acid filtrate (that is, proteose and amino and residual nitrogen) will give the proteose nitrogen in 1 cc. of solution.

The method permits of very close checking, and duplicate analyses were run throughout. The accuracy and critical evaluation of this method will be considered in a separate paper, about to be published. Hiller and Van Slyke (12), in a comprehensive study of certain protein precipitants, also pointed out that trichloracetic acid precipitated only whole protein, permitting amino acids and intermediate products to pass into the filtrate. They emphasized that the acid should be used in concentrations lower than 5 per cent; otherwise there may be some hydrolysis of the protein. It is true that their method called for boiling of the filtrate in order to decompose the trichloracetic acid. Greenwald (13) in 1915 stated that the protein was not dissolved when a concentration as high as 22 per cent trichloracetic acid was used.

In the experiments here reported it was found that 4 cc. of 20 per cent trichloracetic acid (making a final concentration of 13 per cent) served for maximum precipitation. If only 2 cc. were used, further addition of the acid to the filtrate caused more precipitation. The presence of trichloracetic acid did not interfere with the kjeldahl determinations, and therefore it was not deemed necessary to boil the filtrate in order to decompose the acid.

TABLE 1

Activity and percentage nitrogen as amino nitrogen freed from tuberculin protein-X by proteolytic enzymes

NUMBER OF DAYS DIGESTION	PROTEIN + H_2O	PROTEIN + 0.2 PER CENT HCl	PROTEIN + 0.3 PER CENT Na_2CO_3	PROTEIN + GASTRIC JUICE	PROTEIN + TRYPSIN	PROTEIN + 0.3 PER CENT Na_2CO_3 + TRYPSIN	PROTEIN + EREPSIN
	(1 cc. of solution = 0.01 gram protein)						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.60	0.55	0.55	0.83	0.83	1.93	0.83
2	0.61	0.77	0.72	1.22	2.05	2.70	1.58
3	1.16	0.55	0.55	1.45	2.53	2.98	1.77
6	0.70	0.62	0.65	1.53	3.11	3.74	1.54
10	0.83	0.39	0.62	1.95	3.90	4.38	3.26
Skin tests							
4	4+, 4+, 5+, 5+	3+, 4+, 4+	1+, 1+, 3+	tr, tr, tr, tr	2+, 4+, 4+, 5+	tr, tr, −, −	3+, 4+, 5+
	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls tr, tr
10	4+, 5+,	2+, 2+, 4+	3+, 4+, 4+, 5+	tr, − −, −	tr, tr, 2+, 4+	−, −, −, −.	3+, 3+ 5+, 5+
	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls −, −	Controls tr, tr
pH							
1	5.4	3.0	7.4	3.0	5.6	7.4	5.6
10	5.0	3.5	7.6	3.0	5.4	7.6	5.6

Van Slyke (14) stated that phosphotungstic acid precipitated not only protein, proteose and peptone but also some of the diamino acids, while tungstic acid is claimed by Hiller (12) to precipitate protein and protein intermediate products completely without precipitating amino acids. Therefore, parallel determinations with the two acids were run upon the tuberculin protein-X, and the nitrogen in the filtrates proved to be (1)

with phosphotungstic acid, 1.59 per cent N, and (2) with tungstic acid, 1.79 per cent N. The difference between the two is within experimental error, and therefore it will be permissible to designate as proteose the difference between the trichloracetic and phosphotungstic filtrates.

RESULTS

Analyses of one lot of the noncoagulable water-soluble protein-X of tuberculin were as follows:

1. It gave positive protein and amino acid tests, that is, biuret, Millon, vanillin, glyoxylic, ninhydrin and diazo tests.
2. It gave a positive Molisch test.
3. It was a cream-colored powder, and the fraction here studied was non-dialyzable.
4. Total nitrogen = 10.2 per cent (not water and ash-free basis), and of this, whole protein = 50.7 per cent; proteose, 33.8 per cent; amino, 5.9 per cent; and residual, 15.5 per cent.

The first series of experiments was planned to follow the effects of the three different enzymes upon the biological activity of the tuberculin protein-X at the same time that the freeing of amino nitrogen was being followed. As seen in table 1, the change in pH during ten days of digestion at 37°C. was not significant and could not account for the other results observed. For each determination the amount of amino nitrogen in a control sample of the enzyme alone was determined, and subtracted from the total amount of amino nitrogen found in the respective digests, and therefore the nitrogen percentages reported represent only the amino nitrogen freed from the protein.

Discussion of Experiment 1

Water, 0.2 per cent HCl and 0.3 per cent Na_2CO_3 apparently have no hydrolyzing effect upon the protein, since even after ten days of incubation there was no increase of free amino nitrogen in the digest. The skin tests also revealed no significant loss of activity. Pepsin, which is supposed to act by breaking open the whole protein molecule, frees a small amount of amino nitrogen and completely destroys the activity even in four days' digestion.

Trypsin, in neutral solution, frees more amino nitrogen, presumably by its attack upon the proteose, which previous experiments have shown to

be present. The activity of the protein is only slightly decreased after four days' digestion, but after ten days there was a recognizable decrease. If 0.3 per cent Na_2CO_3 is added to the trypsin then the activity of the protein is very quickly and completely destroyed. Erepsin, which attacks only proteose, frees considerable amino nitrogen, but has no effect at all upon the activity of the preparation.

These experiments strongly suggest that loss in activity accompanied attack upon whole protein and that it was not concerned with destruction of proteose. Therefore, further experiments were planned, in which a chemical study of the attack upon whole protein by the method described above, was made, along with the change in activity during digestion.

In the following tables the protein, proteose, amino and residual nitrogen are reported as percentages of total nitrogen. It will be noted occasionally that the sum of the protein, proteose and residual nitrogens does not equal exactly 100 per cent. This error results in the subtraction of the nitrogen values for the enzyme solution itself from the nitrogen values of the protein tested plus the enzyme. In numbers of analyses on small amounts of material errors of a few per cent were unavoidable. The digests were tested only immediately and after five days' incubation.

Table 2 records results showing the effect of water, 0.3 per cent Na_2CO_3 and 0.46 per cent HCl upon the tuberculin protein-X; 0.46 per cent HCl was used because this was found to be the strength of acid in the gastric juice of this series of experiments.

Discussion of Experiments of Table 2

On the whole, compared with the effects due to the different enzymes, there is no significant loss in activity or change in nitrogen partition during five days of digestion of the tuberculin protein-X with H_2O, 0.3 per cent Na_2CO_3 and 0.46 per cent HCl. Minor effects which have been noticed are a slight loss in activity and a slight decrease in whole protein, due to the acid and alkali.

In the case of the water, slightly more whole protein and less proteose were found at the end of five days than in the beginning of the experiment. Although every number had been checked, it seemed that this must be an error and therefore the entire experiment was repeated, with the same result. An explanation would be a synthesis, upon standing, of

whole protein, possibly protean, at the expense of the proteose, or an occlusion of proteose. This point will be studied further.

Discussion of Experiments of Table 3, Which Show the Effect of the Various Enzymes upon Another Lot of Tuberculin Protein-X

If gastric juice is mixed with the protein, and tested immediately, the mixture is active and the nitrogen partition normal. However, after

TABLE 2

Effect of weak acid and alkali upon tuberculin protein-X

SUBSTRATE	TIME OF DIGESTION	TUBERCULOSIS SKIN TESTS	NORMAL SKIN TESTS	NITROGEN PARTITION			
				Protein N per cent	Proteose N per cent	Residual N per cent	Amino N per cent
(1 cc. of solution = 0.01 gram protein)							
H_2O	1 hour	3+, 5+	tr, tr	65.5	32.1	2.4	5.7
	5 days	3+, 4+, 4+, 5+	−, −	71.4	18.2	10.4	7.4
H_2O	1 hour			60.5	26.5	11.8	8.0
	5 days			70.0	14.8	15.1	10.3
Na_2CO_3 = 0.3 per cent	1 hour	4+, 4+, 5+	−, −	64.8	32.0	3.2	7.6
	5 days	2+, 2+, 3+, 4+	−, −	57.6	24.7	17.7	13.6
HCl = 0.46 per cent	½ hour	3+, 4+, 5+	−, −	65.7	24.6	9.7	4.0
	5 days	2+, 2+, 3+	tr, −	57.0	34.3	8.6	10.8

digesting for five days at 37°C. the activity is completely lost, the whole protein is reduced to about one-fifth of the original amount, the proteose is more than doubled, and the residual and amino nitrogen is increased to about the same extent as by the 0.46 per cent HCl. If the gastric juice is boiled before mixing, then no loss of activity occurs, and there is no significant change in whole protein.

Another sample of gastric juice (this time human) gave the same loss in activity and reduction in percentage of whole protein. In this case

there was a marked reduction, instead of increase, in percentage of proteose, and this may be explained on the basis that erepsin could have been

TABLE 3

Effect of enzymes upon tuberculin protein-X

SUBSTRATE	TIME OF DIGESTION	SKIN TESTS IN TUBERCULOUS GUINEA PIGS	SKIN TESTS IN NORMAL GUINEA PIGS	NITROGEN PARTITION			
				Protein N per cent	Proteose N per cent	Residual N per cent	Amino N per cent
(1 cc. of solution = 0.01 gram protein)							
Pepsin: Pawlow-pouch gastric juice	¼ hour	3+, 4+, 4+, 4+	−, −	62.5	39.6	0.0	6.3
	5 days	tr, tr, −, −	−, −	12.3	82.0	5.8	13.1
Pepsin: Boiled, same as above	¼ hour	3+, 4+, 4+, 4+	−, −	58.9	40.6	0.9	4.1
	5 days	3+, 3+, 4+	−, −	52.8	50.9	0.0	5.2
Pepsin: Human gastric juice	1 hour	1+, 2+, 2+	−, −	24.7	61.4	13.8	14.8
	5 days	tr, −	−, −	26.4	41.9	31.7	21.6
Trypsin: 0.1 per cent, Fairchild's	1 hour	3+, 4+ 4+	−, −	46.9	39.6	13.6	16.2
	5 days	4+, 4+ 4+	−, −	40.8	5.7	53.5	35.8
Trypsin: (0.1 per cent) + Na_2CO_3 (0.3 per cent)	½ hour	2+, 4+ 4+	−, −	50.2	31.2	18.5	25.0
	5 days	tr, tr, tr,	tr, −	15.2	24.6	60.0	49.4
Erepsin: Dog intestine	1 hour	3+, 4+, 5+	−, −	72.6	13.6	13.8	11.5
	5 days	4+, 5+, 5+, 5+	1+, 1+	51.6	12.4	35.9	20.0

present in this gastric juice, since it was taken from a man with a gastric ulcer. It is noteworthy that the first tests were made one hour after

mixing, and even within this short period of time almost complete loss in activity occurred and the entire attack upon whole protein had already taken place, demonstrating the explosive nature of pepsin attack upon protein.

With trypsin in neutral solution, as in the former set of experiments, no loss in activity occurred within five days. There was some immediate attack upon whole protein, which, however, was not increased greatly during continued digestion, so that the total percentage of whole protein was not reduced very extensively. The chief attack, as had been assumed in the former experiments, was upon proteose, that is, almost complete destruction, with a corresponding increase in residual and amino nitrogen. When the trypsin is reinforced with 0.3 per cent Na_2CO_3, then protein is more easily attacked and reduced to approximately as low a percentage as by pepsin. Simultaneously, the activity is completely lost.

Erepsin, as postulated before, is not able to reduce whole protein to any marked extent, and similarly does not destroy the activity.

SUMMARY

Whenever the whole protein molecule (noncoagulable water-soluble fraction) is attacked and changed to proteose and amino acids, as by the action of pepsin or trypsin with Na_2CO_3, there is a complete loss in activity of the protein, as determined by negative skin tests in tuberculous guinea pigs. The effect is a direct result of enzymic action, since boiling of the enzyme before mixing destroys its ability to bring about these results.

Trypsin, in neutral solution, attacks first proteose, with no loss in activity.

Erepsin, an enzyme which is not able to attack the whole protein molecule, but which is able to act upon the peptide bonds already free in the proteose fraction, does not cause any loss in activity.

These experiments indicate that the active substance of the water-soluble noncoagulable fraction used in this study is the whole protein molecule, or else it is so closely associated with this protein that its activity is lost when the whole protein molecule is disrupted. More detailed analyses of this protein are in progress in order to determine its exact nature.

Appreciation is expressed to Dr. Milton T. Hanke for his suggestions and interest in this work.

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THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

VI. ACID HYDROLYSIS OF TUBERCULIN[1,2]

ESMOND R. LONG

The thermostability of the active principle of tuberculin has been recognized since the discovery of tuberculin. In the preparation of Old Tuberculin culture medium and bacilli are boiled together for about an hour, after which the bacilli are filtered off and the filtrate is concentrated to one-tenth of its original volume by evaporation at close to the boiling temperature without impairment of activity. This is in sharp contrast to the behavior of the true bacterial toxins, which are destroyed or at least inactivated by boiling.

The stability of the active principle in the presence of acid in the cold has been emphasized before. The experiment described in this article was carried out to determine its stability in the presence of acid at the temperature of superheated steam, and the relation of protein hydrolysis to the loss or persistence of tuberculin activity. The effort was made to select a series of acid concentrations so graduated that beginning as well as advanced hydrolysis of protein could be observed.

A highly potent preparation of tuberculin prepared on the medium described in study I of this series was exactly neutralized with sodium hydrate. The solution remained clear. One sample of 50 cc. (no. 1) was left in the neutral state, and five other samples were acidified as follows with increasing amounts of normal hydrochloric acid: no. 2, 0.5 cc; no. 3, 2.5 cc; no. 4, 5.0 cc; no. 5, 10.0 cc; and no. 6, 25.0 cc. The final concentrations of acid were thus approximately, no. 1, neutral; no. 2, n/100; no. 3, n/20; no. 4, n/10; no. 5, n/6; and no. 6, n/3.

Precipitation of protein following the addition of acid occurred in flasks nos. 3, 4 and 5, being greatest in no. 4. The concentration of acid in no. 4 was presumably near the isoelectric point of some of the protein present.

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

The six samples, distributed in small Erlenmeyer flasks, were then heated in the autoclave at 120° and 14 lbs. pressure for four hours. At the end of this time precipitation was apparent in flasks, nos. 3 and 4, being greater in no. 3. Flasks nos. 1, 2, 4 and 5 were absolutely clear.

All samples were now exactly neutralized with sodium-hydrate solution without filtering. Sodium-chloride solution was then added to each sample in such amount that the final concentration of salt was exactly the same in each flask. Next, distilled water was added in such amount that the volume was the same in all flasks. The samples were thus finally similar in all respects, except for modifications induced by heating with the different concentrations of acid.

Comparison of protein content, proteose content and skin reaction was next made for each sample. To 5 cc. of the contents of each flask 0.5 cc. of 20 per cent trichloracetic acid was added. Precipitation occurred immediately in the first four samples. A trace of precipitate developed in the fifth and a minute trace in the sixth sample on standing.

Ten-cubic centimetre samples of each flask were next treated with 1-cc. amounts of trichloracetic acid, allowed to stand one-half hour, and filtered from the precipitate formed. The clear filtrate was then divided in each case into two parts. One part was saturated with ammonium sulphate and the other part treated with a 10 per cent solution of tannic acid. Precipitates formed in both cases in all samples. If the precipitate which came out on the addition of trichloracetic acid be arbitrarily called "whole protein" and it be assumed that precipitation of whole protein was complete, the precipitate which occurred in the filtrate from

FIG. 1. SKIN REACTIONS IN PATIENT SENSITIVE TO TUBERCULIN, WITH SAMPLES OF TUBERCULIN HEATED IN THE AUTOCLAVE FOUR HOURS AT 120° WITH HCl

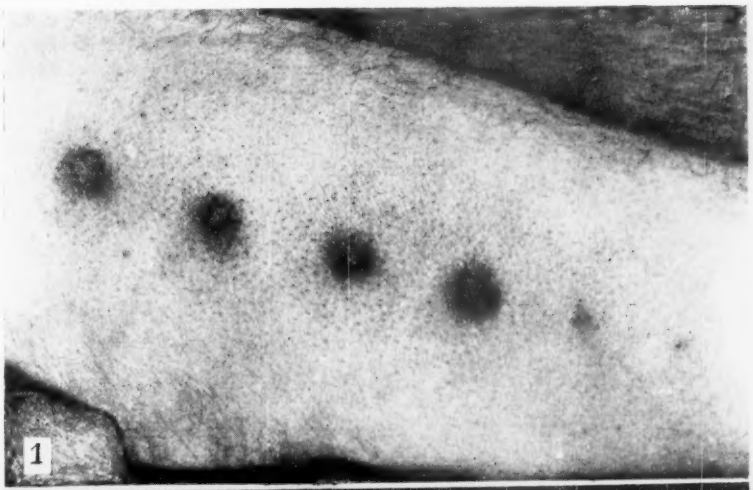
Six samples of the following reactions were autoclaved: no. 1, neutral; no. 2, n/100; no. 3, n/20; no. 4, n/10; no. 5, n/6; no. 6, n/3. After neutralization and adjustment to the same salt concentration and fluid volume, 0.05 cc. from each sample was injected. The figure shows strong reactions with nos. 1, 2, 3 and 4, a trace of reaction with no. 5, and no reaction with no. 6.

FIG. 2. PROTEIN PRECIPITATES IN TUBERCULIN HEATED WITH ACID

The same samples which were injected as described under fig. 1, were treated with trichloracetic acid for the precipitation of protein. Precipitation is marked in nos. 1, 2, 3, and 4. A trace of precipitate has occurred in no. 5. No. 6 is practically clear.

FIG. 3. PROTEOSE PRECIPITATE IN TUBERCULIN HEATED WITH ACID

The same samples which were injected as described under fig. 1, and analyzed for protein as described under fig. 2, after removal of the protein, were treated with 10 per cent tannic acid for the precipitation of proteose. Precipitation occurred promptly in all tubes.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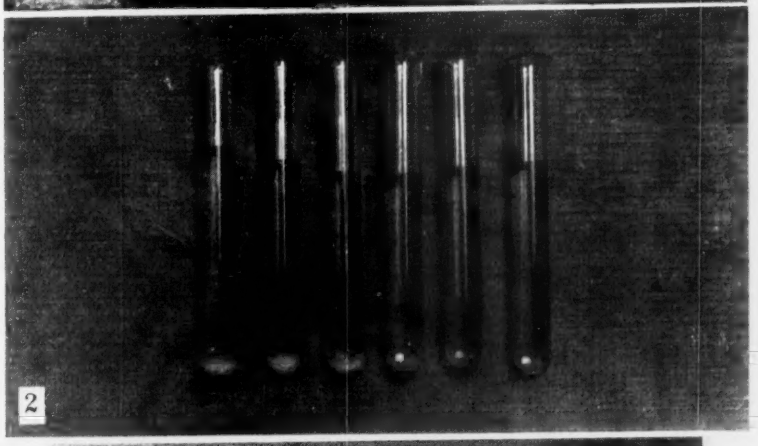
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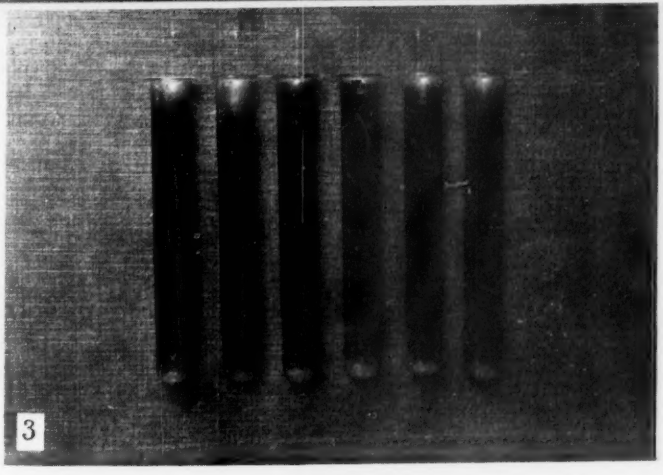
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the whole protein on saturation with ammonium sulphate may be termed "proteose." The bulky precipitate which occurred with tannic acid also represented proteose, plus any peptone present. Only traces of the latter, however, appeared to be contained in the hydrolyzed samples, for on filtration of the ammonium-sulphate precipitate of proteose, the addition of tannic acid caused very little precipitate at any reaction.

Skin tests were then made with (1) the hydrolyzed, unprecipitated samples, (2) the trichloracetic-acid precipitates, and (3) the ammonium-sulphate precipitates in the filtrate from the trichloracetic-acid precipi-

TABLE 1

Acid hydrolysis of tuberculin

Tuberculin heated for four hours at 120° in the following concentrations of hydrochloric acid

	SAMPLE					
	1	2	3	4	5	6
Acid concentration.....	neutral	n/100	n/20	n/10	n/6	n/3

Precipitation of protein and digestion products

	SAMPLE					
	1	2	3	4	5	6
1. Protein (precipitate with trichloracetic acid in neutralized samples)						
Precipitate.....	4+	4+	4+	3+	trace	trace
Skin reaction.....	4+	4+	4+	4+	trace	0
2. Proteose (precipitate on saturation with ammonium sulphate in the filtrate from trichloracetic-acid precipitates)						
Precipitate.....	1+	1+	1+	2+	1+	1+
Skin reaction.....	1+	1+	1+	1+	0	0
3. Peptone (precipitate with tannic acid in the filtrate from ammonium-sulphate precipitates)						
Precipitate.....	trace	trace	trace	trace	trace	trace

tates. Powerful reactions were given with samples nos. 1, 2, 3 and 4 on intracutaneous injections of 0.05 cc. of the hydrolyzed unprecipitated samples in a patient sensitive to tuberculin. The effect was so severe that marked systemic reaction occurred. The patient developed asthma two hours after the injections, and headache, fever and severe chills came on in six hours. The temperature rose from 98.6° to 102.0°. Symptoms subsided in the course of the next day.

Figure 1 shows the skin reactions in this patient. The degree of reaction was about the same in the case of the first four samples. A trace of reaction was given with no. 5, and none whatsoever with no. 6.

The "whole protein" precipitated by trichloracetic acid and the "proteose" precipitated in the filtrate from the trichloracetic-acid precipitate were next injected into the skin of large tuberculous guinea pigs, with a large enough area of skin carefully shaved to permit twelve injections. Powerful reactions were given with the "whole protein" from the first four samples, weak reactions were given with the "proteose" from the first four samples, a trace of reaction was given with the proteose from the fifth sample, and no reaction whatsoever was given with the proteose from no. 6.

Figure 2 shows the trichloracetic-acid precipitates of protein in samples 1, 2, 3 and 4 and the absence of more than a trace of precipitate in samples 5 and 6. Figure 3 shows the proteose precipitates with tannic acid in the filtrate from the trichloracetic acid. All tubes show precipitate. Quantitative differences are not apparent in the figure. These were brought out much better in the ammonium-sulphate precipitate from the trichloracetic acid, and gross comparative nephelometric determinations of the amount of proteose are recorded in table 1, which summarizes the experiment.

DISCUSSION

It is unnecessary to dilate on the fact that no exact boundary-line can be drawn between whole protein and primary proteose. Protein molecules consist of large combinations of amino acids, apparently about 70 per cent of them in the form of a straight chain. Enzyme action, heating with acid, and even prolonged heating in water, split the molecule by hydrolysis at points which are for the most part undetermined. The result is smaller molecules which are in general less readily precipitated than the original whole protein. The process of hydrolysis is unquestionably a progressive one, and at the end of such a process as that described in the preceding pages of this article and in the preceding article, it may be assumed that we have present large molecules, slightly smaller molecules, moderately smaller molecules, and much smaller molecules, all in the same mixture, to use adjectives of admittedly inexact quantitative significance.

We are unacquainted with the precise chemical structure of any of them. It is therefore impossible to settle on a point at which the mole-

cule stops being a protein and becomes the next lower stage in protein disintegration, a proteose. Accordingly it becomes necessary to adopt an arbitrary standard based on physical factors. Solubility in whole or partly saturated salt solutions, and precipitation with acids or heavy metals, are the arbitrary analytical standards usually adopted.

In this investigation I have chosen precipitation by trichloracetic acid as the arbitrary standard for complete precipitation of whole protein. It is commonly assumed to be a whole-protein precipitant in blood analysis. The filtrate from this precipitate still contains substance of general protein nature, which is precipitable by saturation of its solution with ammonium sulphate. It is perfectly possible, in view of the vagueness of boundary between "protein" and "proteose," that others would call all or some of this ammonium-sulphate precipitate "protein" sufficiently soluble not to be precipitated by trichloracetic acid.

The significance of these experiments lies in the fact that only those samples of hydrolyzed tuberculin containing material precipitable by trichloracetic acid gave skin reactions in a tuberculous subject. All samples contained substance not precipitable by trichloracetic acid but precipitable by saturation with ammonium sulphate. It is noteworthy that this material, proteose by arbitrary definition, had tuberculin action only in the first four samples; that is, in just those samples which contained active material precipitable by trichloracetic acid. The proteose precipitate in the first four samples was thus biologically different from that in the last two.

This result could be interpreted as meaning that the active material was all protein and that trichloracetic acid failed to precipitate it entirely, the unprecipitated portion coming down with ammonium sulphate in the next precipitation. However, it does not speak against a hypothesis that activity is a function which persists into the proteose stage in the course of protein disintegration but disappears before what is commonly designated proteose is completely broken up. Fractionation of the "proteose fraction" may settle this point.

SUMMARY

A highly potent tuberculin prepared on synthetic medium was neutralized, and normal hydrochloric acid was added to 50-cc. samples of it in such amounts that final concentrations of the acid were n/100, n/20, n/10, n/6, and n/3. These samples, with one which was neutral, were heated in the autoclave four hours at 120°. They were then neutralized

with sodium hydrate, and to them salt and water were added in such amount that the final volume and salt content were the same in all. The first four samples (beginning with the one heated at neutrality) gave powerful skin reactions in a subject sensitive to tuberculin. A trace of reaction was given with the fifth sample and no reaction with the sixth. Trichloracetic acid gave a precipitate immediately with the first four preparations, a trace of precipitate on standing in the fifth, and a smaller trace with the sixth. The trichloracetic-acid precipitates were filtered off, and all the filtrates were saturated with ammonium sulphate. A precipitate developed in all tubes. The first four precipitates of this series had tuberculin activity, but not the last two. The significance of these results is considered under *Discussion* above. In addition to their bearing on the question of the protein nature of the active principle, the results emphasize the great stability of this principle.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN

VII. THE EVIDENCE THAT THE ACTIVE PRINCIPLE IS A PROTEIN[1,2]

ESMOND R. LONG AND FLORENCE B. SEIBERT

In the literature on the chemical nature of the active principle of tuberculin, argument has been practically confined to the question: Is the active principle a protein or not? A majority of investigators have felt that it is a protein, yet without conclusive evidence. Some have assumed it to be a protein on the theory of probability. It is customary in some laboratories to use a water-extract of dry tubercle bacilli as a standard reagent for producing the tuberculin skin test. Tubercle bacilli are known to be chiefly protein. Furthermore, proteins have been by almost common consent considered the active substances in immunity reactions in general. Hence it seemed safe to attribute the activity of such water-extracts to their content of a specific protein. Baldwin (1) and Krause (2), who have used water-extracts in preference to other forms of tuberculin, because of the uniformity in strength of preparations made by this method, repeatedly refer to "tuberculoprotein" as the cause of the reactions elicited.

The best evidence for the protein nature of the active principle has come from (1) the simultaneous production of the principle eliciting the tuberculin reaction, and of protein, in what were originally nonprotein media, and (2) the destruction of activity by proteolytic enzymes. The chief dissent has rested upon the production of highly active preparations in which protein could not be detected by any of the recognized protein tests. Literature in which some of the opposing claims are given has been reviewed by Long (3).

In general, the methods which have already been used in the study of this problem are still the best at our disposal. However, the methods themselves have been improved, and recent experiments have made it

[1] From the Department of Pathology of the University of Chicago, and the Otho S. A. Sprague Memorial Institute.

[2] Aided by a grant from the National Tuberculosis Association.

possible to avoid certain pitfalls. One source of error, which has probably at times led to incorrect conclusions in previous work, is the extraordinary effect of glycerol on the biuret reaction, noted by Seibert and Long (4). The biuret test, which ordinarily detects proteins in a dilution of 1:5000–10,000, has only one-half to one-fourth this delicacy in preparations containing the quantity of glycerol present in ordinary tuberculins. The active principle of tuberculin is effective in causing a reaction in such minute amount that preparations might be highly active and owe their activity to a protein undetectable by this means.

The experiments recorded in the preceding studies I to VI of this series all point toward a protein nature of the active principle. In brief these may be summarized as follows:

1. The active principle of untreated tuberculin is a colloidal substance which does not pass through animal and vegetable membranes, except occasionally in traces.

2. A colloid giving all the usual protein tests can be precipitated by acids at its isoelectric point, pH 4.0. This substance is highly active in the tuberculin test. Precipitation is not complete by this method, however, and the filtrate, containing some protein, also retains tuberculin activity.

3. Saturation of tuberculin solutions with ammonium sulphate precipitates all detectable protein. The precipitate is the most highly active of all tuberculin preparations we have obtained. The filtrate contains no protein and shows no tuberculin activity whatsoever.

4. The most specific reagents for protein at our disposal, proteolytic enzymes, lessen or destroy tuberculin activity at the same time that they disrupt protein present. As whole protein decreases and protein cleavage products accumulate in solutions of tuberculin subjected to proteolytic enzyme action, tuberculin activity decreases or disappears.

5. Hydrolysis by hydrochloric acid destroys activity only as it destroys protein (substance precipitable by trichloracetic acid). Little hydrolysis of protein and no loss of activity occur in tuberculin heated four hours at 14 lbs. and 120° in the presence of hydrochloric acid concentrations up to n/10. Protein is hydrolyzed to the point where it will not precipitate with trichloracetic acid, and at the same time activity is destroyed, when the same procedure is carried out in the presence of concentrations of hydrochloric acid of n/6 or more.

The results listed above would be conclusive evidence that the active principle is a protein were it not for the notorious capacity of colloids on precipitation to adsorb substances of smaller molecular weight, and the

further possibility of firm chemical union between protein present, which might itself be inert, and a highly active group. The problem is exactly the same as that of the nature of the bacterial toxins, such as diphtheria and tetanus toxins. Bacterial toxins also are colloids which lose their activity under the influence of proteolytic enzymes, and are precipitated by protein precipitants, and come down completely on saturation with ammonium sulphate. Yet they diffuse more rapidly than most proteins, and highly active toxin preparations have been prepared which do not give the ordinary reactions for proteins. This last, however, as Wells (5) states, may merely mean that the toxins are so powerful that they are active in solutions so dilute that they do not respond to protein tests. Wells cites, as an illustration of this possibility, ricin, the toxin of the castor-bean. This was isolated by Osborne, Mendel and Harris (6) in such purity that one-one thousandth of a milligram was a fatal dose per kilo of rabbit. It appeared, moreover, to be inseparably associated with the coagulable protein of the castor-bean, and was believed to be identical with it.

In the case of tuberculin, also, preparations of extraordinary activity have been obtained. One-ten thousandth of a milligram of a product precipitated by ammonium sulphate, injected into the testis of a 400-gram tuberculous guinea pig, is sufficient to destroy all the spermatocytes present and abolish spermatogenesis. This amount could not be detected by any of the ordinary chemical tests, but in larger quantity this material readily gives the biuret, Millon and glyoxylic acid tests which characterize proteins and their cleavage products.

This strongly suggests, if it does not prove, that the substance is of protein nature. However, the coincidence of tuberculin activity and protein color-tests does not rule out the possibility that the activity is due, not to a whole protein, but to a protein cleavage product, containing amino acids identified by the above color tests. Löwenstein and Pick (7) and Jousset (8) have claimed that these protein derivatives of smaller molecular weight than whole protein carried the activity.

A certain amount of our evidence favors this view. In the first place, although, as a rule, active substance did not dialyze from the untreated tuberculin in our experiments, occasionally some activity could be noted in the diffusate, particularly after the substance had been once precipitated. In this respect, again, the active principle of tuberculin resembles the bacterial true toxins. These, although colloidal, pass through ordinary animal and parchment membranes to a slight extent, being said to

diffuse decidedly more rapidly than other proteins and their own antitoxins. Ultimate leakage of the dialyzing sacs was of course to be considered in our experiments, but we believed we could exclude this in most cases. Diffusion occurred most frequently in those preparations which had been subjected to much chemical manipulation. Furthermore, when activity could be detected in the diffusate, protein reactions were given. It seemed possible that we were dealing with a protein containing an active group, and that at times the large protein molecule split with the production of a smaller molecule, still containing the active group, but small enough to pass through the dialyzing membrane.

The fact that a coagulable and noncoagulable protein were present, both of which appeared to be active, was to be considered in this connection. We could not be absolutely sure of the activity of the coagulable fraction, as it was necessary to coagulate it in order to separate it from the other, and in the process of coagulation there was a strong possibility of occlusion of the unquestionably active noncoagulable with the coagulable fraction. We can only say that when the coagulated fraction was thoroughly washed and dissolved or finely suspended in weak alkali, which was later neutralized, strong tuberculin reactions were obtained. The hypothesis was not implausible that we were dealing with one original coagulable protein from which the noncoagulable was derived by beginning hydrolysis, both containing the active group.

On the other hand the experiments on enzymic digestion reported in the preceding article suggest more strongly than any other evidence that the active principle is a whole protein. It has been many times previously reported that proteolytic enzymes destroy the activity of tuberculin, but the parallel changes in the protein molecule have not been noted and measured previously. In experiments described in study VI, in which the noncoagulable fraction was used, it was shown that, as long as most of the protein of the preparation used remained in the form of whole protein molecules, activity was marked. When, however, it was broken up into proteose and amino-acid molecules activity disappeared. Furthermore, the enzyme erepsin, which exerts a profound effect upon the cleavage products of protein, but is inactive on whole protein, failed to affect the activity of the preparation. This is good evidence that the activity does not reside in a small cleavage product. The experiments in which hydrolysis by acids was carried out indicate that, although activity is strongest when whole protein is present, a certain amount of activity persists in the large cleavage products formed in beginning hydrolysis.

We do not consider the subject absolutely settled. We believe, however, that our evidence for the protein nature of the active principle of tuberculin is as well established as that for the phytotoxin ricin, and better established than that for the bacterial true toxins. If, however, the substance is a complete protein, complete, that is, in contradistinction to such proteins as the histones and gelatin, we should expect it to be antigenic in normal animals, and conclusive evidence for this antigenic activity has not yet been presented. Work on this, as well as further experiments on cleavage of the substance, and analysis of our products, are in progress.

SUMMARY

The active principle of tuberculin appears to be a whole protein. The experimental evidence for this is as follows: The active principle of untreated tuberculin is a colloidal substance which does not pass through animal and vegetable membranes, except in traces. A colloid giving all the usual protein tests may be precipitated by acids at its isoelectric point, pH 4.0. This substance is highly active in the tuberculin test. Precipitation of protein is not complete by this method, however, and the filtrate, containing some protein, also retains tuberculin activity. Saturation of tuberculin solutions with ammonium-sulphate precipitates all detectable protein. The precipitate is the most highly active of all tuberculin preparations we have obtained. The filtrate contains no protein, and shows no tuberculin activity whatever. The most specific reagents for protein at our disposal, proteolytic enzymes, lessen or destroy tuberculin activity at the same time that they disrupt protein present. As whole protein decreases and protein cleavage products accumulate in solutions of tuberculin subjected to proteolytic enzyme action, tuberculin activity decreases or disappears. When whole protein disappears on acid hydrolysis activity is lost. As long, however, as whole protein is present activity remains.

There is evidence that a protein product of smaller molecular weight than "whole protein" may be active. Active principle in traces dialyzes. "Proteose" obtained by ammonium-sulphate precipitation, after "whole protein" has been precipitated by trichloracetic acid, has some activity. These results signify that activity is associated primarily with the whole-protein molecule, but persists in spite of a certain amount of disintegration of the molecule.

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STUDIES IN TUBERCULOSIS

VII. ACTIVE PRINCIPLES OF TUBERCULIN PREPARED FROM NONPROTEIN SUBSTRATES[1]

FREDERICK EBERSON[2]

Comparatively little is known about the constitution of tuberculin and the nature of the active principle involved in a tuberculin test. If further light can be thrown on these problems, it might be possbble to interpret the test more accurately, enhance its diagnostic value and explain the mechanism of the reaction.

Diagnostic tuberculins which are used routinely at the present time appear to be unsatisfactory because they lack certain requirements. The ideal preparation should be simple and constant in composition, and should be prepared by a standard method; it should be free from substances which give nonspecific reactions, and these should show reasonable correlation with the extent and severity of the tuberculous infection. Because the interpretation of a tuberculin test depends chiefly upon what is seen in the skin, it is important that there be no extraneous factors modifying the capacity of the skin to react specifically. Since vasomotor excitability appears to run parallel with the intensity of the tuberculin reaction, it is evident that nonspecific proteins or indeterminate irritating substances which affect vasomotor excitability should be excluded.

For these reasons the problem has been studied by using tuberculins prepared from nonprotein synthetic media. Preliminary studies with such material were reported by the writer one year ago (1). These tuberculins contain only elements manufactured by the tubercle bacilli in the nutrient substrate. From this medium it is possible to separate chemically a number of fractions which represent a tuberculin principle more accurately than ordinary complex tuberculins.

The present investigation, a further development of studies begun four years ago, and reported elsewhere (2), includes (A) sensitization experi-

[1] A preliminary report of this work was given at the meeting of the Pacific Coast Branch of the Society of Experimental Biology and Medicine, February 15, 1926.

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ments on normal, nontuberculous guinea pigs, (B) intracutaneous tests on tuberculous guinea pigs, and (C) clinical diagnostic tests with tuberculin fractions in a series of juvenile patients.

PREPARATION OF TUBERCULIN FRACTIONS

Tuberculin was prepared from nonprotein media of the following composition, according to a formula of Gessard and Vaudremer (3):

Ammonium succinate	0.5 gm.
Dipotassium phosphate	0.5 gm.
Magnesium sulphate	0.25 gm.
Calcium chloride	0.125 gm.
Distilled water	100.0 cc.

To one portion of this substrate 2 per cent glycerine was added. After the tubercle bacilli had been growing on this medium for five to six weeks, the tuberculin was prepared according to the usual method by filtration and concentration to one-tenth of the original volume. The growth and staining properties of tubercle bacilli were modified by this synthetic medium. In the glycerinated portion the microörganisms grew more luxuriantly than in the portion to which no glycerine had been added. In the former, half of the microörganisms lost their original acid-fast characteristic, while all of them lost it in the glycerine-free medium.

A weighed volume of tuberculin (100 gm.) was fractionated by adding it to 19 volumes of pure methyl alcohol to effect complete precipitation. The filtrate, after treatment with four volumes of pure ethyl ether, yielded a precipitate containing a second fraction. From the resultant filtrate a third fraction was obtained by concentrating the material at 37°C. to practical dryness. These substances were dissolved separately in a given volume of physiological salt solution, to which 0.25 per cent ether-tricresol was added as a preservative. The alcohol-insoluble fraction represented 4.6 per cent by weight of the original tuberculin, and one cubic centimetre of the test material contained 49.8 mgm. of active substance. The ether-insoluble fraction, 2.3 per cent by weight of the original tuberculin, contained 25.3 mgm. of active substance in one cubic centimetre. The ether-soluble third fraction was exceedingly small in amount, and up to the present time has not been checked with sufficient quantitative accuracy to warrant a definite figure. Roughly, it represented less than 0.5 per cent by weight of the original tuberculin and

contained approximately 4.4 mgm. of active substance in one cubic centimetre. This fraction was gummy and fatty or waxy, and *gave none of the tests for protein.*

A. SENSITIZATION EXPERIMENTS ON NORMAL, NONTUBERCULOUS GUINEA PIGS

Duplicate series of normal guinea pigs weighing 250–300 gm. were injected weekly with gradually increasing amounts of each of the tuberculin fractions by the subcutaneous route. The number of injections totalled ten and ranged from 0.1 cc. for the first dose to 1.2 cc. of the different fractions at the time of the last injection. From the quantitative determinations it was calculated that the animals received the following total amounts of active substances in the course of treatment: alcohol-insoluble fraction, 318.7 mgm.; ether-insoluble fraction, 192.3 mgm.; ether-soluble fraction, 29.5 mgm.

Intracutaneous tests were made one week after the last injection. Homologous as well as heterologous fractions were injected in amounts of 0.01 cc. into the shaved and sterilized skin of the abdomen. The results were read after 24 hours and daily thereafter. The outcome of these experiments was decisive and confirmed the studies which have been alluded to already. Of added significance here is the fact that *protein-free substrates yielded a tuberculin which was active in toto and contained specific sensitizing substances that could be separated out by chemical methods.* One or more injections of derivatives or fractions from ordinary tuberculin or from a nonprotein tuberculin substrate, therefore, sufficed to elicit typical skin reactions in nontuberculous guinea pigs. These results are illustrated in figures 1 to 3. Control animals were uniformly negative with the same substances.

B. INTRACUTANEOUS TESTS WITH TUBERCULIN FRACTIONS ON TUBERCULOUS GUINEA PIGS

In the tuberculized guinea pig it was demonstrated that these same fractions would give unequivocally positive skin reactions by the intracutaneous technique. The results were obtained repeatedly in several duplicate series of animals. Preliminary tests were made after the inguinal lymph nodes had become palpable in animals previously inoculated subcutaneously with a constant amount (1.2 mgm. dry weight) of human

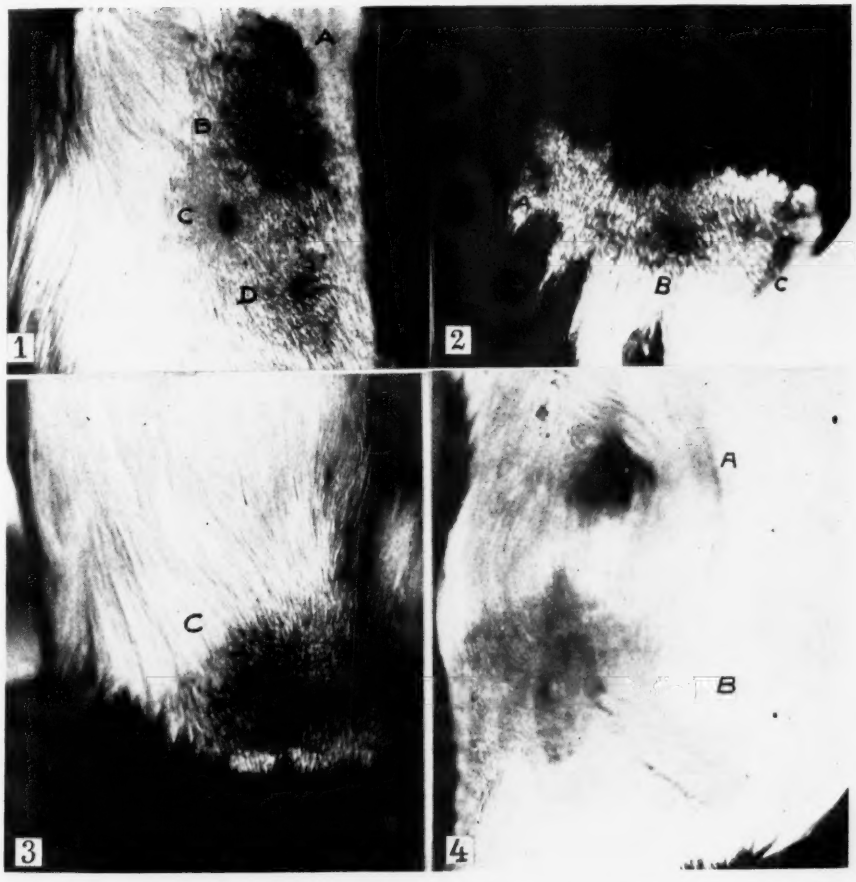


FIG. 1. Intracutaneous test in normal, nontuberculous guinea pig sensitized with ether-soluble fraction from glycerine-free and protein-free tuberculin medium. Twenty-four-hour reading after injection with 0.01 cc. ether-soluble fraction (glycerine-free medium)—*A;* 0.01 cc. ether-soluble fraction (glycerinated medium)—*B;* 0.01 cc. ether-insoluble fraction (glycerinated medium)—*C;* 0.01 cc. original unfractionated tuberculin (glycerinated medium)—*D.*

FIG. 2. Intracutaneous test in normal, nontuberculous guinea pig sensitized with ether-insoluble fraction from glycerinated nonprotein tuberculin medium. Dosage is the same as in fig. 1. Ether-insoluble fraction—*A;* Ether-soluble fraction—*B;* original unfractionated tuberculin—*C.*

FIG. 3. Same guinea pig as in fig. 2, showing the lesion at *C* in natural size.

FIG. 4. Intracutaneous test with 0.01 cc. of ether-insoluble fraction from glycerinated tuberculin medium in a guinea pig which has been inoculated three weeks before with 1.2 mgm. of human tubercle bacilli. Reaction with tuberculin fraction at *B.* A draining tuberculous abscess is shown at *A.*

tubercle bacilli of fixed virulence. In most instances this occurred after three weeks. Each of the test substances was injected in amounts of 0.01 cc. into the skin of the abdomen, and the results were noted after 24, 48 and 72 hours. An illustration of such a reaction with the ether-insoluble fraction is shown in figure 4.

In another group of guinea pigs studies were made to determine how early in the course of a tuberculous infection skin reactions might be observed. The animals were inoculated subcutaneously with 1.5 mgm. of human tuberce bacilli (Saranac strain H55). Three days later intracutaneous tests were made with each of the tuberculin fractions and with ordinary human O.T. Tests were done in duplicate, and the series were additionally controlled by repeating the same injections in normal, non-tuberculous animals. Uniformly positive results were obtained in all of the tuberculous animals at a time when ordinary O. T. elicited no skin reaction. The deductions from these experiments is that synthetic

Description of Figures 5 to 10

FIG. 5. Intracutaneous tests with alcohol-insoluble fraction A (*A*) and alcohol-insoluble fraction B (*B*) in guinea pig inoculated with *B. tuberculosis* 72 hours prior to test. Reaction, 24 hours old, at (*A*) 1.5 x 1.0 cm. with marked reddening, induration and umbilication; (*B*) 1.2 x 1.2 cm. with similar picture.

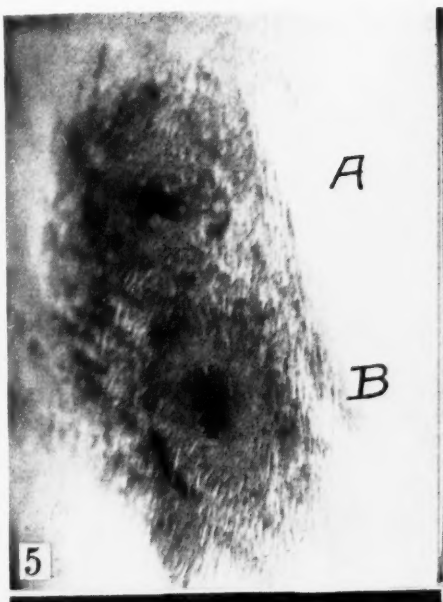
FIG. 6. Intracutaneous tests with ether-soluble fraction A (*A*) and ether-soluble fraction B (*B*) in guinea pig inoculated with *B. tuberculosis* 72 hours prior to test. Reaction, 24 hours old, at (*A*) 0.75 x 0.5 cm. with reddening, induration and central necrosis; (*B*) 0.75 x 0.75 cm. with marked purplish edematous zone and erythema 1.0 cm. in diameter surrounding lesion. (Fraction A represents the glycerine-free substrate and fraction B the glycerinated substrate.)

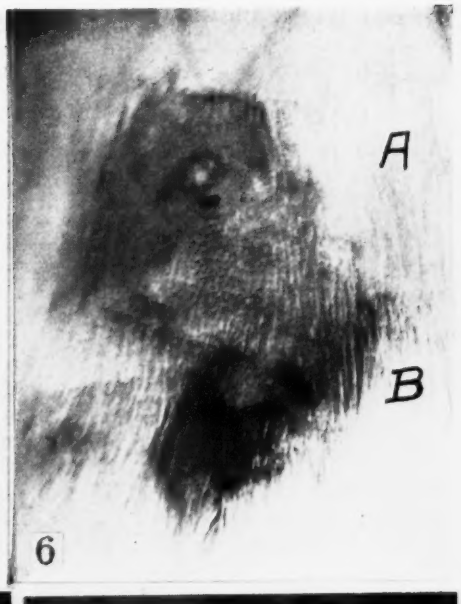
FIG. 7. Intracutaneous test with 0.1 cc. of 1:1,000 dilution of ether-insoluble fractions in patient whose diagnosis was peribronchial tuberculosis. *A*—Fraction prepared from glycerine-free tuberculin. *B*—Fraction prepared from glycerinated tuberculin. Appearance of reactions after 48 hours.

FIG. 8. Intracutaneous test with 0.1 cc. of 1:1,000 dilution of unfractionated tuberculin prepared from protein-free medium in patient whose diagnosis was peribronchial tuberculosis and tuberculosis of the cervical lymph nodes. *A*—Tuberculin from glycerine-free medium. *B*—Tuberculin from glycerinated medium. Appearance of reaction after 72 hours.

FIG. 9. Intracutaneous test with 0.1 cc. of 1:1,000 dilution of ether-soluble fraction (non-protein according to tests) in patient whose diagnosis was peribronchial tuberculosis and tuberculosis of the cervical lymph nodes. *A*—Fraction prepared from glycerine-free tuberculin. *B*—Fraction prepared from glycerinated tuberculin. Appearance of reaction after 72 hours.

FIG. 10. Intracutaneous tests with 0.1 cc. of 1:1,000 dilution of synthetic tuberculin fractions in patient with tuberculosis of both upper lobes. *A*—Ether-insoluble fraction from glycerine-free substrate, 1.5 x 2.0 cm. with marked reddening and induration. *B*—Ether-insoluble fraction from glycerinated substrate, 1.0 x 1.5 cm. with induration and reddening. *C*—Unfractionated tuberculin from glycerinated substrate, 1.5 x 1.0 cm. with induration and reddening. Appearance of reactions after 24 hours.


A
B
5


A
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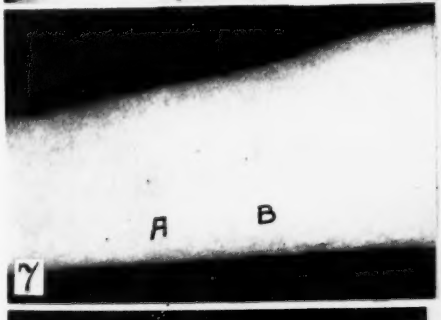
6


A
B
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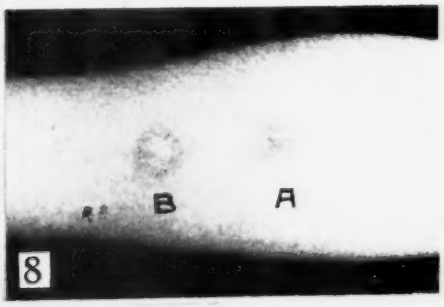
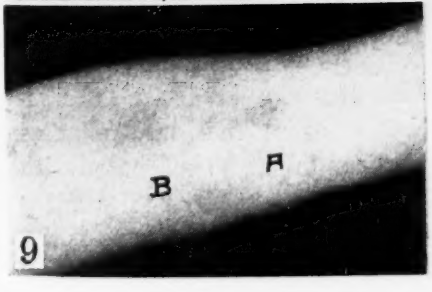
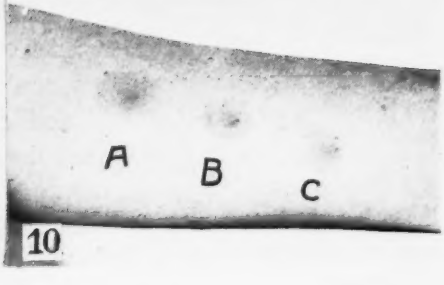

B
A
8


B
A
9


A
B
C
10

tuberculin fractions are specific in guinea pigs as early as three days after inoculation with a moderately virulent strain of tubercle bacillus. A few typical reactions are illustrated in figures 5 and 6, representing tests made with alcohol-insoluble fractions and ether-soluble fractions, respectively.

CLINICAL DIAGNOSTIC TESTS WITH TUBERCULIN FRACTIONS IN CHILDREN

Intracutaneous tests have been made thus far on 150 children and a smaller group of adults. Sixty juvenile patients have been tested with fractions prepared from ordinary tuberculin. The remainder received protein-free substrate tuberculin and its fractions. These preparations, eight in number, included a duplicate series of substances which were derived from glycerine-free tuberculin substrates. Controls were made with ordinary human and bovine tuberculins and with physiological salt solution in each patient. For the intracutaneous tests 0.1 cc. of a 1:1,000 dilution of each test substance was injected. The results were read after 24, 48 and 72 hours. The amount of "tuberculin substance" which was injected differed for each preparation. The fractions contained decidedly less of the active substance in any given dose in comparison with ordinary tuberculin, yet they proved to be potent as well as specific. A few of these reactions are illustrated in figures 7 to 10. The relative amount of active substance in a given test was calculated as follows: ordinary Old Tuberculin, 0.1 mgm.; alcohol-insoluble fraction, 0.005 mgm.; ether-insoluble fraction, 0.0025 mgm.; ether-soluble fraction, 0.0005 mgm.

This study brought out several interesting points. Intracutaneous tests with tuberculin fractions gave definite skin reactions which were usually less severe than those which were observed with ordinary tuberculins. The results, nevertheless, could be better correlated with the clinical histories, physical findings, and with the X-ray. In certain instances the observations suggested that the reactions might be correlated with the degree of activity or of an arrested condition in a given tuberculous infection. However, more data are being analyzed in order to verify this striking observation. Of special interest was a group of contact patients, in a number of whom a clinical diagnosis of tuberculosis could not be made. In these children the ordinary tuberculins gave markedly positive reactions while the fractional tuberculins gave negative reactions.

This was true also in another group of patients who had no positive clinical findings and whose X-ray films showed bronchial adenitis.

Summary of tuberculin tests in a series of 150 children

	per cent
Positive intracutaneous tests with ordinary O. T. and with tuberculin fractions.	70.0
Positive tests with ordinary O. T. and negative tests with tuberculin fractions.	30.0
Positive tests with ordinary O. T. in patients with negative clinical findings and negative X-ray	22.0
Positive tests with tuberculin fractions in patients with negative clinical findings and negative X-ray	00.0

SUMMARY AND CONCLUSIONS

Three fractions have been derived by chemical methods from tuberculins prepared with synthetic nonprotein media. They represent alcohol-insoluble, ether-insoluble and ether-soluble substances, and comprise, respectively, 4.6 per cent, 2.3 per cent and approximately 0.5 per cent by weight of the original tuberculin. The ether-soluble fraction is gummy and fatty or waxy, and gives none of the tests for protein.

In the tuberculous guinea pig the potency and specificity of these tuberculin fractions have been demonstrated as early as three days after inoculation of the animal with B. tuberculosis.

It has been shown for the first time that small amounts of tuberculin fractions prepared from protein-free synthetic substrates are capable of sensitizing normal, nontuberculous guinea pigs. In these animals typical skin reactions can be elicited subsequently by intracutaneous injections of minute amounts of homologous as well as heterologous fractions and of unfractionated tuberculin from which such fractions have been prepared.

Clinical trial of these fractions in juvenile patients has demonstrated for the first time that the substances have diagnostic value. Positive skin tests were perfectly correlated with positive clinical and laboratory findings of tuberculous infection. Such was not the case for ordinary Old Tuberculin in over 20 per cent of young patients in a group of 150 who were studied routinely. In a number of instances the observations suggested that the reactions might be correlated with the degree of activity or with arrested tuberculous infection.

The active substance contained in a dose used for intracutaneous tests in patients was calculated as follows: alcohol-insoluble fraction, 0.005 mgm.; ether-insoluble fraction, 0.0025 mgm.; ether-soluble fraction, 0.0005 mgm.

The present studies point to a method which makes an accurately standardized tuberculin available for routine clinical work. It is believed that the active principle of tuberculin can best be isolated by this or by a similar method.

The observations on sensitization suggest that these may be applied to studies of immunity, and, by extension, to therapeutic studies in the experimental animal. Such work, now in its third year of progress, will be reported in the future.

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ATTEMPTS AT THE ACTIVE IMMUNIZATION OF GUINEA PIGS BY TUBERCULOUS URINARY ANTIGENS

A Preliminary Report

JOHN J. ENRIGHT[1]

At the present time the only specific immunity to tuberculosis that exists generally in the human race is due to the natural haphazard infection that comes to the individual from his environment. The fact that Nature makes use of living tubercle bacilli in the process of immunization has led innumerable investigators to feel that research along such lines holds a great measure of promise in the prevention of tuberculosis.

It has been proved repeatedly that injections of living tubercle bacilli lead to the production of immunity in experimental animals; while, of the very numerous attempts at active immunization by dead bacilli and their products, the most successful have not been able to produce more than a relatively transient immunity.

This state of affairs is responsible for most workers in tuberculosis research accepting as a fact that practical active immunization against tuberculosis is possible only when tubercle is formed in an animal as a result of infection with living bacilli.

Sufficient research work of the proper character has hardly been done to justify us in believing that successful immunization can result only when living tubercle bacilli are used. Eberson (1925) also reports that the innumerable failures of earlier workers to use the different tuberculins successfully in the production of immunity might be attributed to improper technique and to the possibility that due attention has not been given to the important rôle played by solubility, diffusibility and penetration of substances used in this field. Recently, Eberson has reported success in sensitizing guinea pigs by injections of tuberculin modified by the addition of acetyl and benzoyl groups. He thought that the

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chemical change of the tuberculin made it more acceptable from a pharmacological viewpoint.

There is another avenue of attack that should be investigated before it is decided that living tubercle bacilli are necessary for the production of specific tuberculosis immunity. The need for such a study appears from a more detailed consideration of Nature's method of producing immunity. How does it come about that tuberculosis of one lymph node, for instance, is all that is necessary to establish a state of allergy in the general tissues of the usual experimental animals? Of course, a great gap lies in our knowledge of the process, but, nevertheless, this question is considered too infrequently.

The specific sensitizer, when discussed, is quite generally spoken of as a tuberculin, although the evidence to prove this point is scanty. Baldwin (1910) noted that "auto-sensitization is not a sensitization to any product with which we are familiar. It may be a complex product formed by the interaction of the bacilli and tissue cells of the focus." Paterson (1918) termed the sensitizer "the active principle," and others use the term "tuberculo-protein."

The hypothesis on which this study is based is that the special sensitizing substances that are responsible for the production of allergy in the general tissues of the body may be excreted, in an unchanged state, in the urine of active tuberculous patients.

An investigation of whether the concentrated, partly purified urines of active tuberculous patients can be used successfully to immunize guinea pigs should be a prerequisite to the decision that living tubercle bacilli are necessary in the process. Such a study, in its early stages, is the subject of this report.

There is a serious discrepancy of opinion as to the value of the detection of these tuberculous urinary antigens in the diagnosis of tuberculosis, but there is more agreement as to the presence of such antigens in a large percentage of active tuberculous patients. The production of immunity in guinea pigs by the injection of tuberculous urines free from tubercle bacilli would be convincing evidence to those who still doubt the presence of specific tuberculous urinary antigens.

EXPERIMENTAL

The same method of partly purifying the antigen was used as that which we reported in a previous work (1924), involving the use of a collodion sac which will permit diffusion of the inorganic salts and urea from

the concentrated urine and allow the dialyzing residue to contain those substances which are less diffusible than erythro-dextrin. The antigen, indefinite substances called proteic acid, oxy-proteic acid, etc., and the chromo-proteins will remain in the sac.

The urines that were subjected to concentration were the pooled urines of several active tuberculous patients. A special effort was made to obtain day specimens from ambulant patients who were having moderate exercise, and also to exclude urines that indicated any renal disease. The concentration was done *in vacuo* at a temperature of less than 65°C., and was 1/20 of the original volume of the urine. After the concentrated urine was cooled to about 5°C., it was filtered through paper, and the filtrate then placed in a collodion sac to dialyze in running water. Dialysis continued until tests showed that the dialyzing residue contained neither chlorides nor urea. The residue was then concentrated *in vacuo* to 1/20 of the original volume of urine. This product was finally filtered through a Mandler porcelain filter to make certain that no bacilli remained in the preparation.

The following method was used to estimate roughly the amount of antigen in the product: Five-hundredths (0.05) cc. of the material was injected intracutaneously into the arms of three allergic individuals next to similar quantities of Old Tuberculin in the different dilutions 1/10,000, 1/1,000, 1/500, and 1/100. From this study it was seen that the antigenic preparation caused areas of induration and reddening to the same extent as the 1/500 dilution of Old Tuberculin.

Fifteen healthy male guinea pigs of approximately the same ages and weights were placed in separate cages in a special animal house. Five of these were used as controls; the others received seven weekly injections of the urinary antigen, subcutaneously, just above the right inguinal region. On successive weeks 0.1, 0.3, 0.5, 0.8, 1.0, 1.0, and 1.0 cc. of antigen, respectively, were injected. The weights of all guinea pigs were noted at the time of each injection. On the day after the last urinary injections 0.05 cc. of a dilute homogeneous suspension of a virulent strain of tubercle bacilli was injected intracutaneously into all 15 guinea pigs, injections in five being at the site of the urinary injections, and in the other five on the opposite side of the abdomen. This division of the questionably protected group of animals into two subgroups was made because Baldwin (1911), Bessau (1916), Selter (1921), and others who have produced allergy by dead bacilli or their products have found that sensitization is only local and not general. The intracutaneous method

of injection of the living tubercle bacilli was used, so that, as in the Koch phenomenon experiments, the progress of the local disease could be observed daily.

It was fully realized that the number of important variable factors entering the investigation emphatically demanded large series of animals, but due to the radical nature of the study preliminary experimentation on series of five guinea pigs in a group was chosen. As it is practically impossible to use equal doses of tubercle bacilli, and guinea pigs with equal reacting capacities to the urinary antigens as well as to the living tubercle bacilli, it is of the greatest necessity to use large series of animals in each group to "absorb" the variable factors.

The value of the use of urinary antigens in immunizing guinea pigs or, at least the answer to the question, whether study on larger series

TABLE 1

Showing the number of days that elapsed between infection and death

GUINEA PIG	GROUP A: NORMAL GUINEA PIGS RECEIVED 0.05 CC. OF DILUTE SUSPENSION OF LIVING TUBERCLE BACILLI (CONTROLS)	GROUP B: RECEIVED SAME DOSE AS GROUP A AT SAME SITES AS INJECTIONS OF URINARY ANTIGENS	GROUP C: RECEIVED SAME DOSE AS GROUP A AT SIDE OPPOSITE INJECTIONS OF URINARY ANTIGENS
	days	*days*	*days*
1	73	64	85
2	64	63	120
3	144	73	166
4	71	162	178
5	78	72	135
Average length of life after infection.....	86	87	137

was indicated, was judged by the local reaction to the living bacilli, the length of life in the different groups of guinea pigs, and the amount of gross pathological changes seen at the autopsies.

RESULTS

It must be noted immediately that the observations of the local reactions to tubercle bacilli gave no indication that any allergy was present in the tissues but for the following weak exception: Contrary to what might be expected, definite nodular formation and ulceration were observed in four of the five guinea pigs in that group that received the injections of bacilli on the side of the abdomen opposite to the immuniz-

ing doses on an average of 5 to 7 days earlier than in the other groups. However, in none of the groups was there any sign of an early tuberculin-like reaction such as that which follows the intracutaneous injection of tubercle bacilli into guinea pigs immunized by inoculation with living tubercle bacilli (see Krause (1920)). There was, however, a slight tendency toward healing in the superficial ulcers of those animals that lived 120 or more days after injection, that is, even in one of the control guinea pigs.

As to the periods that had elapsed between infection and death of the different guinea pigs, table 1 will show the results clearly.

It is seen from the data recorded in table 1 that there is one guinea pig in each group whose period of illness proved to be a major exception to the otherwise fairly uniform results of that group. Guinea pig 3 of group A and 4 of group B lived about twice as long as other animals of these groups, while no. 1 of group C lived little more than half as long as the others of its own series.

How to interpret the results recorded in table 1 is the all-important question. Are we justified in averaging the number of days in each group, and come to the conclusion that we have prolonged the lives of the guinea pigs in group C an average of 50 days, due to the injections of the urinary antigens? The writer thinks not. The reasons will be discussed in the latter part of this report.

The autopsies exhibited interesting features. Four out of five animals in group A, the control, as well as in group B, those receiving the immunizing injections at the same point as the infection, died about 9 or 10 weeks after infection. The guinea pigs of group A dying at this time showed, grossly, an extensive generalized tuberculosis, including advanced disease of the spleen, liver and lungs. On the other hand, three of the four guinea pigs of group B dying at this same period, in addition to the guinea pig in group C that died in 85 days, had practically the same disease as group-A animals, except that no tubercles could be found in the lungs. However, the other group-B animal had roughly the same disease as the four previously mentioned group-A guinea pigs.

The four animals of group C and one in each of group A and group B that lived for over 17 weeks after infection had very advanced tuberculosis of the spleen, liver and lungs, and also superficial ulcers showing a very slight tendency toward healing. No tubercles of the kidneys were seen in any of the guinea pigs.

DISCUSSION

It has been our experience, as well as that of others, that the reactive capacity of the tissues of different guinea pigs to living tubercle bacilli has been a variable factor, or perhaps the variation has been due partly to the impossibility of giving doses equal only within certain poorly definable limits.

These variable factors plus the small number of guinea pigs in each series are responsible for our belief that very little weight should be attributed to results recorded in table 1. This is so, further, because the results of groups B and C are as they are, and not reversed. If the lives of the animals of group B were prolonged, a logical explanation could be given. We would look upon such a result as further evidence that with anything but living tubercle bacilli antituberculosis immunity is a local affair and of very weak intensity. No attempt to explain the results in group-C animals will be made until it is determined whether they can be corroborated on a larger scale.

The writer believes that serious consideration is due any experimental work designed to increase our knowledge of protective inoculation against tuberculosis when the vaccine consists of something other than living bacilli, even if the work should not be proceeding from a logical hypothesis. The methods of Calmette (1921) (1924) and others who use living, even though avirulent, tubercle bacilli, successfully to bring about protection in animals will probably not receive the approval of many authorities, when these methods are advocated for other than experimental purposes. Recently Webb (1925) has pointed out that the use of living tubercle bacilli, even though apparently avirulent, is a dangerous procedure because variation of the characteristics is a prominent feature of all living microörganisms.

It is true that our present knowledge of tuberculosis immunology would lead us to believe that no potent and lasting allergy could be produced without actual infection. Nevertheless, the effects of proving the efficacy of some unquestionably harmless product in giving protection to human beings and cattle are of such extraordinary importance that works similar in nature to those of Eberson should be encouraged.

CONCLUSIONS

The attempts to immunize guinea pigs actively with partly purified tuberculous urinary antigens will have to be made on a scale large enough

to rule out definitely the element of chance before it is desirable to make any interpretation of results.

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THE BACTERICIDAL ACTION OF ULTRAVIOLET LIGHT ON THE TUBERCLE BACILLUS

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The extensive and successful use of the mercury-quartz-vapor light in the treatment of cutaneous forms of tuberculosis and the inability to definitely explain the good results obtained have prompted this work. Various explanations have been offered, but any proof of a bactericidal action is lacking. Mayer and Dworski (1), at the Saranac Laboratory, have concluded from their studies that the ultraviolet rays are bactericidal for the tubercle bacillus after a three-minute exposure at a distance of five inches from the burner.

All the rays of the spectrum possess light, heat and chemical properties, but the chief chemical action is attributed to the violet and ultraviolet rays. Numerous workers, among whom may be mentioned Blunt and Downes (2), Browning, Russ, Bayne-Jones and Van der Lingen (3), have proved the bactericidal action of ultraviolet rays on various microörganisms, but none offers definite proof of a bactericidal action on the tubercle bacillus prior to the work of Mayer and Dworski.

It has been shown that with ultraviolet rays the bactericidal effect increases with the diminution of the wave-length and the increased frequency of the vibrations. Changes in the temperature of the surrounding medium of the microörganisms may slightly alter the bactericidal action. Henri and Cernovodeanu (4) have stated that the bactericidal action of light is independent of temperature. Becquerel (5), however, found that at the temperature of liquid air about six hours were required to kill anthrax spores and the spores of some other fungi with the quartz lamp. The great difference in the time required for the bactericidal action of light at the two temperatures suggests, at least, that the reaction proceeds more slowly at a very low temperature than at a higher one. Changes in the H-ion concentration of the medium probably play no part in the bactericidal action. However, Bayne-Jones and Van der Lingen claim that a lethal effect is accelerated in an

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acid medium. Henri has shown that ultraviolet rays can render the tubercle bacillus non-acid-fast. While this phenomenon would probably not be bactericidal to the tubercle bacillus, certainly the latter's pathogenicity would be greatly diminished, and it would be rendered a more ready victim to the body's defenses. There is probably some selective action of different wave-lengths for special bacteria. Bayne-Jones and Van der Lingen have concluded this from their work; however, there is still some debate along this line.

My aim in this work was to ascertain whether mercury-quartz-vapor irradiation is bactericidal to the tubercle bacillus, without attempting to explain any such action that it might possess. It has been my purpose to study the action of ultraviolet radiation on suspensions of tubercle bacilli, which have been exposed to the radiations for varying periods of time at varying distances. These suspensions of tubercle bacilli, after exposure, were injected into guinea pigs. Skin tests of these guinea pigs were made ten, twenty and thirty days, respectively, after injection of the suspensions. Thirty-five days after inoculation all the remaining guinea pigs were killed, and examined both macroscopically and microscopically for evidences of tuberculous infection. A careful control of the series, consisting of four guinea pigs which were inoculated with untreated portions of the suspension of tubercle bacilli, was kept. It will be noted that 2 of the 24 guinea pigs used died during the experimental period; neither showed evidence of tuberculous infection in any organ or tissue. In order that a very pathogenic variety of tubercle bacilli would be used in the experiment, these were secured from the sputum of persons having advanced pulmonary tuberculosis. This sputum was emulsified in saline solution, slowly centrifugated to throw down the residue of the sputum and any clumps of bacteria which might be present. The suspension was then concentrated until each high-power microscopic field contained from 12 to 16 tubercle bacilli. This suspension was then divided into portions of one cubic centimetre each. These portions were exposed to the radiations of a standard mercury-quartz-vapor lamp, which has been used several times daily during the past five months. In order to obtain radiations of maximum intensity, the lamp was burned ten minutes before the first exposure of the suspension. The portions of the suspension were placed in large watch-glasses, which were surrounded by water to prevent desiccation during the exposure. Immediately after exposure the suspensions were injected intracutaneously into the abdominal wall of the guinea pigs. The exposures and

inoculations were made on November 11, 1925, and the guinea pigs were killed on December 21, 1925.

As stated above, all guinea pigs were killed thirty-five days after inoculation and examined both grossly and microscopically for evidences

TABLE 1

Ultraviolet irradiation of suspensions of tubercle bacilli

GUINEA PIG	TIME OF EXPOSURE OF BACILLI TO RADIATIONS AND DISTANCE OF EXPOSURE	SKIN TEST AFTER 10 DAYS	SKIN TEST AFTER 20 DAYS	SKIN TEST AFTER 30 DAYS
1	Controls; no exposure	−	+	+
2		+	+	+
3		−	+	+
4		−	+	+
5	1 minute at 6 inches	−	+	+
6		−	+	+
7		−	−	+
8		−	+	+
9	3 minutes at 6 inches	−	−	+
10		−	−	+
11		−	−	+
12		−	+	+
13	1 minute at 10 inches	−*		
14		+	+	+
15		−	+	+
16		+	+	+
17	3 minutes at 10 inches	−	−	+
18		−	−	+
19		−	+	+
20		−	−	−
21	5 minutes at 10 inches	−	−	−
22		−	−	−†
23		−	−	−
24		−	−	−

* Died of pneumonia on the 13th day; no evidence of tuberculosis.

† Died of pneumonia on the 32nd day; no evidence of tuberculosis.

of tuberculosis. Findings were as follows: Guinea pigs 1 to 4 inclusive (controls) showed a severe generalized miliary tuberculosis. Guinea pigs 5 to 8 inclusive showed miliary tubercles in the liver and spleen macroscopically, and in the liver, spleen and lymph nodes microscopically.

Guinea pigs 9 to 12 inclusive showed no gross evidence of tuberculosis but microscopic examination revealed tuberculous areas in the liver, spleen and lymph nodes. Guinea pigs 14, 15 and 16 showed a definite miliary tuberculosis which was apparently of the same severity as the infection in the control guinea pigs. Guinea pigs 17 to 20 inclusive

TABLE 2

Ultraviolet irradiations of suspensions of tubercle bacilli

GUINEA PIGS	TIME AND DISTANCES OF EXPOSURES	GROSS OR MICROSCOPIC EVIDENCE OF TUBERCULOUS INFECTION
1, 2, 3, 4	Controls; no exposure	Definite miliary tuberculosis
5, 6, 7, 8	1 minute at 6 inches	Positive
9, 10, 11, 12	3 minutes at 6 inches	Positive
13*, 14, 15, 16	1 minute at 10 inches	Positive (severity of infection apparently same as in controls)
17, 18, 19, 20	3 minutes at 10 inches	Positive
21*, 22, 23, 24	5 minutes at 10 inches	Negative

* Died during the experimental period.

showed a few tubercles in the liver grossly and definite tuberculosis in the liver, spleen and lymph nodes microscopically. One member of this group (no. 18) had a caseous tubercle at the site of injection 2 cm. in diameter. Guinea pigs 21 to 24 inclusive showed no evidence of tuberculosis in any organ or tissue of the body after careful examination, both grossly and microscopically.

SUMMARY AND CONCLUSIONS

1. It has been shown by many workers that ultraviolet light is bactericidal to various microörganisms, but Mayer and Dworski are the only ones who claim to have shown its bactericidal properties for tubercle bacilli heretofore.

2. Changes in the temperature of the surrounding medium of the microörganisms may slightly alter the bactericidal action.

3. Changes in the H-ion concentration of the medium probably plays no part in the bactericidal action.

4. The tubercle bacillus can be rendered non-acid-fast by ultraviolet light. This might explain some of the good results secured in the treatment of tuberculous infections with the quartz lamp.

5. From the results obtained in this experiment it is concluded that the irradiations of the mercury-quartz-vapor lamp used are bactericidal for the tubercle bacillus after an exposure of five minutes at a distance of ten inches from the burner. No attempt is made to explain this bactericidal action.

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THE RADIO AND THE TUBERCULOSIS PATIENT[1]

WILLIAM H. ROSENAU[2]

Is the radio an unqualified boon to the sick? While it is admitted that its advent has contributed much to the happiness, contentment and amusement of innumerable sick patients, has lightened greatly the burden of invalidism and has brought the outside world to the bedside, the question forced upon us is, "Has the radio in some instances a deterrent influence on the well-being of the sick?"

One cannot deny that to many the radio has banished an intense impatience at the delay of getting well, boredom, maladaptation to the outlook of continued invalidism, etc., and successfully substituted contentment, satisfaction, interest in existence, and a proper adaptation to existing unfortunate circumstances.

The effects of the use of the radio are not, however, uniformly favorable. One must, therefore, critically consider those reactions injurious to the patient which result from the use of this great invention. My experience has taught me that consent to the use of the radio should not be given indiscriminately, but that each patient should be individualized.

Outstanding have been the following facts: Many patients who spend hours hunting for distant stations ("D-x hounds"), straining ears and keeping nervously tense in order to catch the indistinct announcements, react unfavorably following such activity. Many are those who are made intensely nervous by listening through ear-phones when loud speakers are not available. The temptation to delay turning out the lights for the night is yielded to by many sick ones. Reports of patients turning on lights after having been prepared for the night, and listening in until wee hours of the morning, are not uncommon. Some patients become extremely weak, nervous, and cold and clammy after listening to the radio, but have no similar unfavorable reactions following the use of the phonograph.

Two types of radio offerings are commonly followed by unfavorable

[1]From the Cragmor Sanatorium, Colorado Springs, Colorado.

[2]Banning, California.

reactions in tuberculous patients. In both types the reaction is due to the overstimulation of the patient with the production of great excitement. The broadcasting of plays of intense situational interest, particularly of the melodramatic character, many times will leave the sick listener nervously overwrought, unable to sleep, and greatly excited. The detailed broadcasting of competitive events, such as football and baseball games, will, in many instances, completely upset a patient who previously had been making excellent progress.

The striking comparison between those patients at the Cragmor Sanatorium at Colorado Springs, who "listened in" to the broadcasting of the recent baseball world series and those who did not, brought out the following interesting and important facts:

1. Patients who were interested in the outcome of the world-series games, but did not "listen in," did not have any untoward symptoms and signs that were encountered in many of the group who used the radio to follow the progress of the games.
2. A small proportion of the patients who followed the games with the radio (5 cases or 25 per cent) did not have any resulting unfavorable reactions. Most of these patients belonged to the nontoxic arrested type of tuberculosis, who had taken long and intensive periods of rest followed by graduated exercise and increasing liberties.
3. The larger percentage (15 cases or 75 per cent) of those who followed the games by radio had definite unfavorable reactions of varying degrees, traceable only to the excitement attendant upon the listening to this competitive event.

Unfavorable Reactions Observed

1. Rise in body temperature:

A: Relative rise, in those patients who had slight rise in temperature above normal limits before the onset of the series, was observed.

B: Rise in temperature of patients who had been afebrile before the onset of the world series.

Case 1: Normal temperature for two months, 98.3°F. before game, 99.8°F. immediately after the game. Because of this unfavorable reaction the patient was forbidden the use of the radio, but the rise in temperature continued for over a week. Another interesting example is as follows:

Case 2: A patient with pulmonary tuberculosis, who had had normal temperature for six months, developed an afternoon rise in temperature up to 99.8°F. during the world series which fell to normal immediately after the completion of the games.

The patients voluntarily gave the information regarding this febrile reaction. In practically every febrile reaction, two or three days after the completion of the games the temperature was again within the normal limits. The highest rise in temperature following such activity in patients, in whom the temperature had been normal previous to this event, was 99.8°F. Many of these patients had definite sensations of unusual warmth during and following the game.

2. Rise in pulse-rate:

A: In every case the pulse showed a definite rise following the listening period and a higher pulse-rate persisting from hours to days after the games. The intense situational tension in many of the games explains much of this increase in pulse-rate.

Case 3. Pulse before the game 84, immediately after the game 148, two hours later 90. This case gave the greatest rise in pulse-rate encountered. All degrees of increase in the pulse-rate below this level were noted.

3. Effect on the rest hour and sleep:

Most cases were unable to sleep during the rest hour and many slept less well during the night.

4. Effect on the appetite and gastrointestinal symptoms:

A number of the patients suffered from some anorexia during this period. Nausea and diarrhea were in some cases met with.

5. Effect on the cough:

A few patients experienced an increase in cough and one patient coughed for several hours continuously following the last game.

6. Sputum:

With the increase in cough a slight relative increase in sputum was observed.

7. Pulmonary haemorrhages:

Two cases had pulmonary haemorrhages; *case 1*, two days after initial rise in temperature and abstinence from radio the onset of a pulmonary haemorrhage occurred; *case 2* had a definite rise in temperature after listening to the games and one week after the end of the series he developed a pulmonary haemorrhage.

Whether this excitement of "listening in" during this period might have played a rôle in the production of the pulmonary haemorrhages is of speculative interest. It may well be a case of *post hoc ergo propter hoc*.

8. Increased nervousness, sweating, weakness, and other signs of unfavorable constitutional effects were noted.

SUMMARY

These observations cannot and should not be ignored. The importance in the treatment of tuberculosis of rest, relaxation, repose, avoidance and prevention of worry, and isolation from excitement and irritation is recognized by all. There can be no doubt that the indiscriminate and unrestricted use of the radio for the patient afflicted with tuberculosis is most unwise. The duty of the attending physician is evident. The reaction of each individual patient following the use of the radio should be carefully noted. Those cases which react unfavorably should not be allowed its use. The patient should be discouraged in the logging of many stations. He should content himself with easily available reception. He should be told to avoid "listening in" to exciting plays and the broadcasting of competitive games in which he is keenly interested. If these precautions are taken, the radio will remain a boon to the sick, and not an unfavorable inhibiting influence to convalescence.

ERRORS IN THE DIAGNOSIS OF TUBERCULOSIS IN CHILDREN[1]

WILLIAM E. CARROLL[2]

This paper is based upon a review of 606 cases discharged from the Meriden Sanatorium (Connecticut) during the past five years. The ages of the patients varied from seven months to fifteen years. No attempt will be made to consider methods of treatment or results obtained. Attention will be given solely to the diagnostic aspect of tuberculosis in children, with special reference to conditions which have been confused with tuberculosis.

In the table appended the major diagnosis only is considered in patients who presented no tuberculous lesion. There were, of course, many patients who, in addition to well established and easily demonstrable tuberculosis of one kind or another, also had chronic hypertrophic tonsillitis, otitis media, blepharitis, corneal ulcers, phlyctenular conjunctivitis, pleurisy with or without effusion, rickets, dental caries, etc. However, when one of these conditions appears in the table it constitutes the major diagnosis and almost invariably the sole cause for impairment of health. With this limitation in mind the table is presented. Diseases in general are arranged alphabetically except that, for the sake of convenience, all bone tuberculosis has been grouped together, and a number of conditions which occurred infrequently appear under the head of *Miscellaneous*, where each condition, however, is listed in alphabetical order. Under the heading *No Diagnosis* are included patients removed within forty-eight hours after admission upon whom there was insufficient opportunity for assembling data.

Diagnosis on discharge

Abscess, pulmonary	2	Miscellaneous	60
Adenitis, nontuberculous	57	Anaphylaxis, foreign protein	3
Bronchiectasis	7	Anemia, secondary	1
Cardiac disease, valvular	6	Appendicitis, chronic	1

[1] Read at a Conference of the Connecticut State Tuberculosis Commission, Meriden, Connecticut, November 2, 1925.

[2] Undercliff, Meriden, Connecticut.

Empyema, pyogenic		10
Obsolete	7	
With sinus	2	
Communicating with bronchus	1	
No disease		48
No diagnosis		12
Osteomyelitis, pyogenic		12
Otitis, media, chronic		12
Pleurisy, (obsolete)		5
Adhesive	1	
Thickened pleura	4	
Pleurisy with effusion		6
Pneumonia, broncho		2
Pneumonia, lobar		2
Central	1	
Unresolved	1	
Postpneumonic, chronic pulmonary disease		3
Rickets		6
Sepsis, oral		11
Tonsillitis, recurring		17
Tuberculosis, bone		51
Ankle	2	
Dactylitis	6	
Hip	19	
Knee	2	
Spine	22	
Tuberculosis, lymph-node		57
Tuberculosis, meningeal		3
Tuberculosis, miliary disseminated		5
Tuberculosis, peritoneal		10
Tuberculosis, pulmonary		197
Juvenile, hilar	40	
Juvenile, parenchymal	71	
Juvenile, parenchymal and hilar	18	
Parenchymal (adult type)	67	
Peribronchial	1	
Undernutrition		30

Arthritis, hypertrophic	1
Ascariasis	1
Asymmetry, chest	1
Banti's disease	2
Blepharitis, chronic	1
Bronchitis, acute	3
Bronchitis, chronic	3
Cholecystitis, chronic	1
Chorea	3
Conjunctivitis, phlyctenular	1
Coxa, vara	1
Cyst, femur	1
Deafness	1
Eczema	2
Endocarditis, rheumatic	1
Epilepsy, minor	2
Fistula, bronchial	1
Hydrocephalus	1
Hypernephroma	1
Hypothyroidism	1
Impetigo contagiosa	1
Influenza, respiratory	3
Lipodystrophy	1
Malaria, aestivo-autumnal	1
Mastoiditis, chronic	1
Meningo-encephalitis	1
Nephritis, chronic, interstitial	1
Nephritis, chronic, parenchymatous	1
Paralysis, infantile	1
Paralysis (birth)	1
Paraplegia, spastic	1
Pericarditis with effusion	1
Pharyngitis, chronic	1
Pyelitis, acute	1
Scoliosis, spine	2
Sinus, branchial	1
Sprengel's deformity	1
Status lymphaticus	1
Syphilis, congenital	3
Ulcers, corneal	1
Vomiting, cyclic	1

The total number of patients discharged is 606; for our present purposes the 12 included under no diagnosis had better be excluded. This leaves 594 patients with completed records. Of these 323, or 54.4 per cent, had tuberculosis in one form or another, while 271, or 45.6 per cent, had no clinical tuberculosis.

It would appear at first glance that there was an enormous percentage

of inaccuracy in diagnosis. This, however, is more apparent than real, for the simple reason that many patients were purposely mislabelled "tuberculous" for the sake of having them admitted, or they were recommended for admission because, even though in good health, they were exposed at home to great danger of infection from tuberculous parents. In the first group are included most of the cases of pyogenic osteomyelitis, rickets, otitis media, draining empyema, and valvular cardiac disease. Such cases are difficult to care for at home, and are not welcome in general hospitals because of their chronicity. They do not, as a rule, offer any problem in diagnosis, although one case of rickets listed was confused with Pott's disease, They are sent to the tuberculosis sanatorium for the sake of providing refuge for them and because all, except the "cardiacs" and the patients with osteomyelitis with sequestra, will do well under sanatorium regimen and heliotherapy. When no sequestrum is present chronic pyogenic osteomyetlitis will also do well. In the second group are included the 48 patients who had no demonstrable disease. The 30 patients included under the head of *Undernutrition* came from very poor economic surroundings. They had no actual disease and suffered from insufficiency of food. They were probably recommended for admission because they were potentially good material for tuberculous infection and would profit greatly by residence at the sanatorium.

There were 6 patients who presented no other involvement than pleurisy with effusion. This condition is usually considered evidence of real or potential tuberculosis. It has been stated that a high percentage of patients with pleural effusion eventually present undoubted evidence of pulmonary tuberculosis. The patients under consideration resorbed their effusions rapidly and, although continued in residence for two years, never showed recurrence of effusions, evidence of a parenchymal lesion or any impairment of health. These effusions did not follow lobar or bronchopneumonia and were not associated with rheumatic endocarditis or arthritis. It will no doubt be freely admitted that these patients were entitled to sanatorium care and to prolonged observation.

The total number of cases considered above is 123. With these excluded there remain 148, or 24.9 per cent, of the total number of discharged patients in whom there was evidently error in diagnosis.

The largest single group was 57 cases of nontuberculous adenitis. The adenopathy in these cases was due to recent acute infections, to carious teeth, to pediculi capitis, etc. It disappeared rapidly with correction of

the primary cause. Adenitis associated with chronically infected tonsils or chronic otitis media is not included in this group.

There were 2 cases of pulmonary abscess. Patients with this condition always have been and probably always will be admitted to tuberculosis sanatoria, sometimes under mistaken diagnoses and sometimes for the sake of providing them with shelter and care. The history of the case, usually traceable to aspiration pneumonia, to septic embolism, or even to lobar pneumonia, the periodic filling and emptying of the abscess, the absence of tubercle bacilli in the presence of copious sputum containing elastic tissue, and the X-ray findings, usually determine the diagnosis when the patient is properly investigated.

A very interesting group may be included under the general head of postpneumonic (including influenzal pneumonia) chronic pulmonary disease. Our cases of bronchiectasis, pulmonary cirrhosis with or without adhesive pleurisy, and of postinfluenzal chronic pneumonitis fall into this group. The cases noted as obsolete pleurisy were also traceable to antecedent pneumonia. These postpneumonic conditions have been discussed in a previous paper (1). It is enough to say here that the patients are in good general condition, their physical signs are more manifest than their symptoms, their sputum even though copious is persistently negative for tubercle bacilli, and they show no progressive deterioration of health. A point to be noted is that clubbed fingers in children are rare in tuberculosis except in the presence of widespread disease with cavitation and great prostration. In children in good general condition clubbed fingers should put one on the look-out for bronchiectasis, chronic empyema, or chronic pulmonary abscess which drains well, and does not cause toxemia.

The 17 cases of tonsillitis with recurrent attacks deserve particular mention. They were admitted chiefly because of pallor, occasional cough, and failure to gain weight. Careful history showed that they had no habitual cough and what cough they did have was associated with sore throat. Most of the children or their parents admitted several attacks of "sore throat and headache" during the course of a year. On physical examination they showed no abnormality except enlarged and scarred tonsils, sometimes with palpable lymph nodes at the angle of the jaw. Repeated physical examination, X-ray investigation and sputum examination gave no evidence of pulmonary disease. In none of them could a diagnosis of tuberculous adenitis be justified. They all had had repeated attacks of tonsillitis, varying from four to ten a year for

each patient. These were apparently ordinary recurring follicular tonsillitis. None gave evidence of Vincent's angina. If a patient has six attacks a year he is ill about one-eighth of the year, and scarcely recovers from one attack when another is thrust upon him. Is it any wonder that he is pale and ill nourished? It is a grievous error to label these patients tuberculous. The stigma of the disease is placed upon them, their education is subject to interference and delay, and their parents suffer undue anxiety. In all instances the diagnosis was manifest from the history. In none was there the slightest evidence of tuberculosis. Their only hope for return to normal health lay in tonsillectomy. This is not a plea for wholesale or indiscriminate tonsillectomy, but certainly the patient who has four, six or eight attacks of tonsillitis a year should have removed an organ which serves only as a depot of infection.

Eleven patients presented no condition other than extensive dental caries, some with and some without pyorrhea, apical abscesses, etc. They improved rapidly under proper dental care. Why they should have been considered tuberculous is not known unless the associated adenitis was the cause of confusion.

Differentiation between prolonged simple bronchopneumonia and tuberculous bronchopneumonia is, of course, difficult. In the two cases under observation here the negative Pirquet test and the absence of wasting inclined us toward the diagnosis of a nontuberculous condition. The ultimate complete recovery of the patients justified the conclusion.

The patient with unresolved lobar pneumonia was sent in from a general hospital, where he had had a typical pneumonia of the right lower lobe. At the time of admission here he was afebrile and felt well, but presented physical signs of consolidation over the lobe involved. These disappeared in about one month, and the patient was apparently none the worse for his experience. The single instance of central pneumonia occurred as an intercurrent infection in a patient under observation for suspected hilar tuberculosis. It is included merely for the sake of completeness and did not offer a problem in differential diagnosis.

The mere recital of the conditions included under miscellaneous disease is interesting. It shows that almost any impairment of health in a child can be regarded as tuberculous, sometimes with, but more often without good reason. It is obvious, of course, that most of the errors were due to insufficient investigation or to utter lack of investigation—to "snap" diagnosis. It surely is a great injustice to diagnose tuberculosis in an ordinary acute respiratory infection from which the patient

will recover in a few days. Even so, 3 patients with influenza and 3 with transient acute bronchitis were admitted. Why clearly defined conditions, such as Banti's disease, chorea, rheumatic endocarditis, chronic nephritis, or acute pyelitis should be confused with tuberculosis is almost incomprehensible if any systematic attempt at diagnosis were made. The same is true of cases of paroxysmal dyspnoea due to foreign protein anaphylaxis.

Recurring appendicitis is confused with tuberculosis because of loss of weight and gastrointestinal disturbances. The children under our observation apparently had a number of slight attacks at home. Their parents did not seek medical advice. The children were found below weight at nutritional clinics and were recommended for admission on general principles. After admission, definite attacks of appendicitis made the diagnosis obvious.

The cases of coxa vara and cyst of the femur were confused with tuberculosis of the hip. Careful physical examination and X-ray investigation would have determined the diagnosis. The hypernephroma was thought to be vertebral tuberculosis because of pain and limitation of motion. The palpable mass was interpreted as a paravertebral abscess. The diagnosis was established at operation.

Lipodystrophy is a rare condition, in which there is great loss of subcutaneous tissue on the face, arms and torso, but little or none on the lower extremities. The girl in question was diagnosed as tuberculous because of her appearance—the face was almost cadaveric—but she presented no physical or X-ray signs of the disease or symptoms of tuberculous toxemia.

Untreated or insufficiently treated tertian or quartan malaria or aestivo-autumnal malaria should be considered in any long-continued fever. The patient in question had the latter type of infection and yielded promptly to quinine therapy. Entire absence of physical signs of tuberculosis, in the presence of continued fever, anorexia, loss of weight and general malaise, raised suspicion that the tubercle bacillus was not the causative factor. The malarial parasite was found in the blood. The spleen was not enlarged.

Pericarditis with effusion, especially a small or moderate effusion, can almost exactly simulate the early symptoms of tuberculosis. Ambulatory cases are not uncommon. Slight, irregular fever, tachycardia, fatigue, pallor and listlessness suggest hilar tuberculosis, even in the absence of physical signs in the lungs. Diagnosis rests upon perception

of the physical signs of effusion and proper interpretation of X-ray findings. In voluminous effusions there is much less danger of error. They must, however, be distinguished from pleural effusions.

There should be no reason for confusing a branchial sinus with tuberculous cervical adenitis. The conditions have little in common except that both occur in the neck.

The patient with Sprengel's deformity was thought to have tuberculosis of the spine because of iregularity in the outline of the back due to the elevated scapulae. Spinal scoliosis is sometimes diagnosed as vertebral tuberculosis on cursory examination. One child with post-infantile foot-drop and paralysis of the quadriceps was admitted as tuberculosis of the hip because of her limp. The case of spastic paraplegia was a compression myelitis due to dorsal Pott's disease, but was wrongly diagnosed as tuberculosis of the hip because of limp due to muscle weakness, incoördination and inability to raise the left foot. The boy had adductor spasm, greatly exaggerated knee-jerks and violent ankle clonus.

Congenital syphilis is only too common. It should be considered in obscure conditions in childhood, whether or not the patient presents stigmata of the disease. One patient had subcutaneous gummata which were considered tuberculous adenitis. The others had no leading indication of syphilis but did have four-plus Wassermann reactions.

Corneal ulcers, phlyctenular conjunctivitis, chronic blepharitis, eczematoid skin lesions are, of course, often manifestations of tuberculosis, especially if several of them occur in the same patient. However, they are very often of simple origin, and the occurrence of any one of them in a patient otherwise healthy and with negative physical and X-ray findings would scarcely warrant the diagnosis of tuberculosis.

The other conditions listed do not require special mention. Either they were manifest on admission or defined themselves after a period of observation.

Of the patients in whom the diagnosis of tuberculosis was established, 197 had pulmonary tuberculosis of one type or another, 51 had tuberculosis of the bones or joints, and 75 had other forms of extrapulmonary tuberculosis. The two latter groups caused little or no difficulty in diagnosis. They all presented well-defined pictures of the condition named. The only question was to decide between tuberculous and syphilitic dactylitis.

In the pulmonary group 67 had frank parenchymal tuberculosis of the

adult type, with unmistakable physical signs, X-ray findings and toxic symptoms. Forty-five had positive sputum, far-advanced disease and a hopeless prognosis. We have had only one patient with positive sputum who recovered. This boy had a lesion limited to the left apex. The remaining 21 patients had lesions limited to one lobe, usually to one apex, and progressed to satisfactory arrest. These patients presented no difficulty in diagnosis.

The chief diagnostic problem centered about juvenile, pulmonary tuberculosis of the nonmanifest type,—hilum disease, peribronchial tuberculosis and early parenchymal involvement without râles. There have been a few patients who presented a complete picture—definite history of exposure, positive Mantoux test, cough, loss of weight, pallor, listlessness, moderate fever, tachycardia, paravertebral or parasternal dulness, roughened inspiration or granular inspiration over the same areas, D'Espine's sign, undoubtedly abnormal hilum shadows or bronchial markings and entire absence of evidence of disease other than tuberculosis which could produce the symptoms.

Many of the patients presumably in this class were recommended for admission because of one or two leading symptoms, or the diagnosis was made solely on X-ray findings, or physical signs were reported which could not be corroborated. In border-line cases the greatest care and discretion must be exercised. The physician is placed in the awkward dilemma of delaying diagnosis when tuberculosis is present, or of stampeding the patient into a disease which he does not have. In spite of all that has been written, the fact remains that the diagnosis of nonmanifest pulmonary tuberculosis, in most instances, must be made as much by exclusion as by any positive findings. There should be a high index of suspicion for other causes of prolonged ill health. Some of these can simulate tuberculosis very closely; others can produce two or three symptoms. Among the former are chronic disease of the upper respiratory passages and accessory sinuses, pericarditis with effusion, low-grade pyelitis and obscure sepsis. Among the latter we have encountered chiefly underfeeding because of poor economic conditions, recurring tonsillitis, foreign protein anaphylaxis, and a condition arising from the overzealous attention of neurotic parents, in which the child exaggerates minor symptoms and refuses to eat because he enjoys being the centre of excitement, or he may retire into negativism as a protest or defense against the harassing to which his parents, with the best intentions in the world, subject him.

With such conditions excluded, a minimum standard of positive evidence should be required. Physical signs may or may not be present; at most they will be slight, such as interscapular or parasternal dulness or impairment at an apex, change in the inspiratory murmur, and sometimes D'Espine's sign. In the absence of physical signs there should be unquestionable abnormality in the hilum shadow or lung markings as shown by X-ray. The tuberculin skin test should be positive, unless rendered temporarily negative by recent measles, pertussis or scarlet fever. With findings such as these in a child continuously below par, and with other conditions excluded, the diagnosis of tuberculosis would be justified. Since we are considering the least possible evidence on which diagnosis could be based a history of definite exposure is not included. It is evident that a great many of the children admitted here did not conform with this minimal standard. Many of the children were well and healthy; others were suffering from nontuberculous conditions.

It is inadvisable to make a diagnosis of hilum tuberculosis or peribronchial tuberculosis from X-ray findings alone. The films should be interpreted in relation with the history and symptoms.

CONCLUSIONS

Too much cannot be said in favor of early diagnosis of tuberculosis. However, in our zeal for early diagnosis we should take every care and precaution not to "railroad" the patient into a disease which he does not have. Difficulty arises chiefly in two groups of patients, namely, those who present very few abnormal signs and those who present a great many abnormal signs. In the former group the diagnosis of tuberculosis should not be made unless it is obvious that the patient is suffering from some impairment of health, and then only after other causes have been carefully excluded. In a sturdy, thriving child the diagnosis of clinical tuberculosis is not warranted, even if he presents physical and X-ray findings which would justify the diagnosis in a child in poor health. Symptoms are more important than signs.

In children who present a great many abnormal physical signs and who, even so, are in good general condition,—afebrile, well nourished, strong,—some condition other than tuberculosis is probably the causative factor. The same may be said of the child with cough of years' duration who remains in good general condition and of the child with clubbed fingers whose general health leaves little to be desired. It must be remembered that bronchiectasis, chronic adhesive pleurisy, pulmonary cirrhosis and chronic bronchopneumonic infiltrations are by no means

uncommon in children. It is not true that lower-lobe tuberculosis in children is relatively frequent compared with upper-lobe lesions. It may be taken as a safe general rule that when the upper lobes are clear a basal lesion is not tuberculous unless tubercle bacilli can be demonstrated in the sputum.

It would seem that definite clinical entities, other than chronic nontuberculous pulmonary disease, are at times confused with tuberculosis. At least, such has been true in 10 per cent of the patients discharged from this sanatorium.

It is not by any means so great an economic disaster to falsely diagnose tuberculosis in a child as it is in an adult who is the wage-earner of a family. It is, in fact, justifiable at times to put a child under antituberculosis regimen on well-founded suspicion of the disease. However, every care should be taken that the child should not needlessly be separated from his family or suffer delay in his education. There is no occasion for haste in diagnosis. It is much better to delay opinion during a reasonable period of observation than to hurriedly make a diagnosis which may prove untrue. In this connection there is one more point to emphasize, and attention cannot be directed to it too urgently. The fact that a child has once been a patient in a tuberculosis sanatorium is not, of itself, *prima facie* evidence that he is tuberculous. It has been our experience that it is almost an even chance that he is not. Even so, many children, who after discharge fall ill with some transient disease, are hastily recommended for readmission. One boy was admitted eight years ago convalescent from a thoracotomy for postpneumonic empyema. He improved rapidly, the wound healed and he was discharged in excellent condition, but had impaired resonance and distant breath-sounds below the angle of the scapula because of thickened pleura. He has since been readmitted twice, each time because of transient cough due to simple acute bronchitis which disappeared in a few days. On each occasion the boy lost seriously in school and his parents were needlessly thrown into panic worrying about him. There are many similar instances. Should this review avail to decrease their incidence it will have served its purpose.

Forty-five and six-tenths per cent of patients discharged from this sanatorium were nontuberculous. In 20.7 per cent there was good cause of one kind or another for recommending the patient for admission. In 24.9 per cent the patient was admitted under gross error in diagnosis.

REFERENCE

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TUBERCULOUS CERVICAL ADENITIS[1]

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While it is perhaps true that little or nothing new can be said of tuberculous adenitis of cervical lymph nodes, yet as a subject it remains as intriguing as it was when Shakespeare gave it mention as the "King's Evil" in the *Tragedy of Macbeth*, and sufferers with the affliction now search as assiduously for a cure as they did in the days of Edward the Confessor, when the royal touch was implored for its alleviation and cure.

As to the significance of enlarged cervical lymph nodes it has been said with equal dogmatism in different eras that they are the "outward expression of wretched humors of the soul" and that they are definite indications, in children, of immunity against pulmonary tuberculosis.

Certain statisticians have observed that no individual, with scars of the neck indicative of tuberculous cervical adenitis in childhood, ever came to autopsy as a result of pulmonary tuberculosis, whereas equally eminent authors have stated that all tuberculous infection enters the body and gains haven first in the lymphatic system and gives rise to the primary tuberculous lesion in the lymph nodes, which may or may not be evidenced by symptoms, and later spreads to other parts of the body.

The frequency of occurrence of enlarged cervical lymph nodes as a result of tuberculosis has likewise been the subject of much conflicting testimony, for one prominent author stated in 1895 that "Even before the discovery of the bacillus of tuberculosis by Koch in 1882 it was generally conceded that hypertrophied cervical lymph nodes known as scrofula was a true tuberculosis of lymphatic glands;" the intimation from this being that all cervical lymph-node enlargement was considered tuberculous.

On the other hand, a more recent writer, with a wealth of experience from which to draw deductions, asserts positively that "Careful examination of children attending dispensaries shows that between 50 and 75 per cent have palpable cervical glands," but that "most of them are due

[1] Read at a monthly Medical Conference of the Connecticut State Tuberculosis Commission at Undercliff Children's Sanatorium, Meriden, Connecticut.

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to carious teeth, hypertrophied tonsils, adenoids, stomatatis, eczema, or pediculi of the scalp, etc.," and that the number due to tuberculosis is by no means as large as has formerly been thought.

Discounting the very natural changes in ideas and theories that have resulted from better knowledge gained by painstaking research and observation, it has at the same time been intensely interesting to search the writings of a few of the modern internists, surgeons and tuberculosis experts with the intent to ascertain their beliefs concerning the significance and meaning of enlarged cervical lymph nodes in relation to etiology, tuberculogenesis, tuberculo-immunity, tuberculo-therapy, etc.

Tyson (1), writing in 1895, stated that the "tubercle bacillus usually attacks the glands nearest its point of entrance, and presumably the cervical glands are infected by bacilli which enter by the way of the nasal or naso-pharyngeal passages." Discussing the significance of the enlarged lymph nodes he wrote, "In former times scrofula was regarded as a protection against consumption but at the present day it is looked upon as a menace because of the danger of systemic infection through it, and it is said that three-fourths of the cases of acute tuberculosis owe their existence to it." He further stated that cases of recovery from tuberculous lymphadenitis in childhood must be regarded as instances of survival of the fittest, and he insisted upon the complete eradication of the local condition.

Cornet (2) in 1907 said

> Tuberculous lymph-adenitis is not for us, as it was for our predecessors, the localized expression of a constitutional anomaly, of a diathesis, but the visible evidence of an infection with tubercle bacilli. We should, therefore, invariably regard the drainage area of the glands earliest invaded as the site of invasion of the virus, excepting only those rare cases in which there is centrifugal progression of the disease.

A more recent communication of Cornet's, however, presents it as his opinion, that "scrofula is a product of mixed infection with pyogenic and tuberculous organisms."

Fishberg (3) asserts that

> Scrofula may be looked upon as a manifestation of the defense reaction of the lymphatic child against a superimposed tuberculous infection. The lymphatic constitution is the preliminary condition in the clinical picture of scrofula; it is the primary or predisposing cause. When in such a child tuber-

culous infection takes place the glandular manifestations of the disease result. Moreover, it appears that scrofulous children only rarely develop pulmonary tuberculosis in later life, and when a lung lesion does occur it runs a very mild course and tends to cicatrization.

W. Williams (4) writing in the *British Medical Journal* in 1921, corroborates this last statement with the following remark:

A very constant, if not invariable fact I have observed in my fifty years experience of tuberculosis is that those members of families with inherited predisposition to the disease who suffer from cervical gland lesion leading to suppuration do not get pulmonary tuberculosis, or indeed any other form of the malady, whereas several other members of the same families without gland lesions and often more robust individuals, frequently develop consumption.

These few extracts concerning the significance of the condition are only an infinitesimal part of the wealth of literature which exists upon the subject, and stand out perhaps only because they are the crystallized expressions of the thoughts which existed in the several decades when each was written.

That the present decade may bring out even more concrete and relevant matter is a development to be hoped for, and its possibility cannot be denied. Indeed, who can say that the heretics will not, in light of the last statement quoted, insist that every child be induced to harbor lymphatic tuberculosis, so that it may, *ipso facto*, become relatively immune to the ravages of phthisis.

Logically, a consideration of the significance of enlarged cervical lymph nodes must needs lead to a more than passing investigation of the phenomena which occur in the lymph nodes as a result of infection with tubercle bacilli.

In accordance with the statements of such pathologists as Coplin, Garrish and others, it may be said that when tubercle bacilli in sufficient numbers are drained into the cervical lymph nodes from such points of entry as the nasopharynx, tonsils, pharynx, or other adjacent structures, the evidence of infection begins. There is quickly developed an epithelioid accumulation resulting perhaps from the proliferation of the endothelial cells; to these are added a few polymorphonuclear leucocytes; all of which, with the accumulation of mononuclear cells apparently derived from the blood, constitute the specific histological tubercle. A number of these may become confluent and the vascular channels become

closed. The affected nodes are, of course, enlarged and are not at first attached to the surrounding tissue. The cellular proliferation continues, and increasingly blocks the vascular supply, and the nourishment of the nodule is more and more curtailed. The toxins of the tubercle bacilli acting upon this area, in which nutrition is greatly impaired, produce coagulation necrosis or *caseation*. When the area becomes partly liquefied, caseous or curdy pus is produced, and the lesion may then be recognized as a tuberculous abscess.

The content of tuberculous abscesses has long been a subject for controversy. Nicholas Senn (5), in his *Principles of Surgery* published in 1901, stated that it was a settled fact that the bacillus of tuberculosis was not a pyogenic microbe, and that in the absence of other bacteria it produced a specific form of chronic inflammation that invariably terminated in the formation of granulation tissue, and that, when true suppuration occurred in the tuberculous area, it occurred in consequence of secondary infection with pyogenic bacteria. It is now held by some that a genuine tuberculous abscess is not an abscess at all, owing to the fact that it does not contain true pus, but does contain the degenerated products of tuberculous inflammation. This material, while it somewhat resembles pus when viewed with the naked eye, when examined with the microscope is found not to contain genuine pus elements, and it has also been repeatedly demonstrated (by staining, by cultures and by inoculation) that pyogenic bacteria are not present. In spite of this, however, there still remain adherents of the idea that liquefaction in a tuberculous abscess may be accomplished only by pus-producing microörganisms, and in spite of the fact that our modern knowledge of allergy has amply demonstrated that tubercle bacilli are necrotizing for the tissues of tuberculous individuals.

The inner surface of the abscess is lined with tuberculous granulations and is known as Volkmann's membrane. We are told that the bacilli in the abscess do not multiply and soon perish in the fluid, although a few living ones may at times be found, and while they exist in numbers in Volkmann's membrane one may fail utterly in growing the bacteria from the pus which has been retained and encapsulated. The series of phenomena may be interrupted at any point by the bactericidal action of the lymphoid structures, including the leucocytes, and the process may terminate by the bacilli succumbing or becoming surrounded by a cordon of leucocytes which prevent extension, and the result will be a fibroid, caseous or calcareous node and represent a "healed in" tuberculosis or a

quiescent tuberculous lymphadenitis. This "healing in" of the affected tissues does not represent a cure but merely a quiescent infection which may, when the protective power is weakened by some intercurrent or secondary malady, break out anew.

Having touched, after a fashion, upon the significance and development of tuberculous cervical adenitis, we arrive therefore at a point where we find ourselves confronted with the necessity of having in mind some method for the treatment of tuberculous abscess, and likewise a procedure which will supply therapeutic anticipation for the potential dangers residing in the "healed in" or latent lesion.

If it is true that enlarged cervical lymph nodes are indicators of immunity to pulmonary tuberculosis, mention of such suggestion having already been made, it may be well perhaps for us to justify ourselves for instituting any treatment for the condition. Certainly this is no task if we bear in mind that the "healed in" lesions are latent foci of tuberculosis, merely awaiting a lessened bodily resistance to break through their imprisoning shells, and spread and disseminate infection to surrounding or even distant structures. Nor must we forget the attendant symptoms frequently occurring with the condition which require alleviation, such as emaciation, fever, night-sweats, anemia, anorexia, etc., and we must remember that children suffering with glandular disease, by very reason of this lymphatic disturbance, are very susceptible to acute endemic infectious diseases, and, when attacked by these, especially measles, whooping cough, diphtheria, etc., the prognosis is most serious.

The development of tuberculous abscess must likewise receive assiduous attention, since its spontaneous rupture is not only liable to produce ugly disfiguring scars, but the rupture may be attended by a wide distribution of the disease throughout the body with resulting acute tuberculosis and death.

The literature no less abounds with descriptions of methods of treatment than it is filled with discussions of the subjects already mentioned; and, as there have been shifting and changes of beliefs as to the significance of the condition, so has there also been continual revision of opinion as to treatment.

We may still observe upon individuals in the street the hideous results that have been achieved by the surgeon who insisted upon complete extirpation of the entire chain of cervical lymph nodes, and likewise we may also still see the horrid scars which have remained as a consequence of a routine of *laissez faire* in the case of suppurating cervical lymph nodes.

The King's Touch and ultraradical surgery now occupy the same degree of value and importance insofar as the treatment of cervical adenitis is concerned, both having been retired after equally nonsuccessful reigns.

Other treatments, too numerous to mention, have from time to time been brought forth to strut their brief hours upon the stage, but if we except the methods which have embraced in their routine careful attention to betterment of environment, food and bodily hygiene, but few plans have had little to offer either of interest or lasting value.

To discuss all of the modes and methods of treatment advanced for tuberculous adenitis would be merely time-consuming, and would not render information sufficiently important to warrant such a digression.

There are at present five important procedures that are being used in attempts to alleviate or cure the disease, and these five methods have been the subjects of more or less wide scientific investigations and discussions. They are as follows:

1. Tuberculin
2. Surgery
3. Roentgentherapy
4. Heliotherapy
5. Puncture and injection, or the Calot treatment

TUBERCULIN

Since the discovery of tuberculin by Koch various investigators all over the world have by many and various methods, and by utilizing many and various forms of the substance, endeavored to use it as a curative agent for lymphatic tuberculosis. Many have reported remarkable results with it, but many, many more have given it thorough trial and discarded it. Actually strict search of the records and the compilation of available statistics may convince the open-minded investigator that there is a dearth of evidence in favor of its use. It is true that upon occasion a wonderful account of its efficiency and value may be read but we cannot say that it is a method of treatment which is effective in producing more recoveries than would ordinarily be anticipated.

SURGERY

The value of surgery in the treatment of tuberculous adenitis is a mooted question, but we who are not given ordinarly to regarding the

condition as a surgical one must heartily agree with the conservative ideas of no less a surgeon than Lahey (6), who states, "We feel that if prompt and active treatment could be instituted and adhered to in cases as soon as they manifest this condition, together with adequate attention to unsatisfactory conditions of tonsils or teeth, and also to other contributing factors, radical surgical measures would rarely be necessary."

Lahey further states that, in his opinion, "Surgery should not be resorted to in tuberculous adenitis until remedial measures, such as attention to infecting foci, improvement of hygiene, and X-ray therapy, have been tried for a considerable period of time,—from six months to a year."

He points out that surgical removal of the lymph nodes is to be considered only after these or other justifiable measures have been tried out with no appreciable result, and with conservative instincts still uppermost he says that "we believe that whenever neck dissection for a not necessarily fatal condition is undertaken one should have definitely in mind the possibility of spinal accessory paralysis, and that paralysis is a serious one."

With these opinions quoted, it seems hardly necessary to discuss further surgical treatment, except to summarize by saying that it is our belief that careful attention to and long application of well-recognized nonsurgical procedures should be maintained before a final decision is reached that any given case requires surgical intervention. It should also be borne in mind that hygiene, and environmental and dietary principles deserve as close adherence with the surgical as with the nonsurgical plans.

X-RAYS

Reports concerning the use of the X-ray as a therapeutic agent in tuberculous lymphadenitis have been so far comparatively meagre, but such reports as have been published have been consistently favorable.

Berry (7) has written that, although no definite conclusions can be drawn from published statistics, it seems that X-ray treatment, efficiently applied, will yield highly satisfactory results. He states that the size and frequency of the dose can only be determined for each individual case. Generally speaking, most workers agree that large doses at intervals of about three weeks, or smaller doses at shorter intervals, are possible alternate plans.

Trostler (8) has reported that, when no liquefaction or breaking down

has occurred, early and brilliant results are the rule and may be expected in 80 per cent of cases. He suggests that lymph nodes that have undergone liquefaction be aspirated and then receive X-ray treatment. He likewise advises that weekly injections of equal parts of phenol and iodine into the diseased lymph node, at the same time continuing the raying, are a useful and efficient adjunct.

Simic (9) enthusiastically reports that, because the X-ray diminishes the virulence of tubercle bacilli, and because of its accurate dosage, greater ease of employment and ability to be used at any time, and because of quicker results and fewer residua, it is unquestionably the procedure of choice.

With all fairness to these observers and granting the importance of their valuable observations, we who have not yet had personal experience with the method do not feel at this time that this plan of treatment has been established on a sufficiently definite basis of specificity to warrant the discontinuance of other methods that are giving almost if not equally good results.

HELIOTHERAPY

Rollier (10) has stated that medical men the world over have lately shown a quickened interest in sun-cure, because it is a method that has proved itself efficacious in the treatment, not only of lymphatic tuberculosis, but in other forms of the disease as well. From his vast experience in the 34 sun-cure institutions with which he is connected, the oldest of which was established in 1903, he has this to say:

> Heliotherapy is the preëminently successful treatment for cervical adenitis, which is always cured without leaving any scars, by one of the three different processes: melting, generally followed by aspiration, spontaneous resorption or sclerosis. The two first mentioned processes occur more frequently than the third. It is seldom, and only in the treatment of adenitis with sclerosis, that we have recourse to the association of X-ray therapy with heliotherapy.

The technique of heliotherapy is now too well known to need description or further discussion. It will suffice to say that those who are using this method of treatment are in the majority of instances following the exposure plan of Rollier.

The results obtained by the proper and careful employment of sun-cure are, for the most part, excellent indeed. The majority of cases of tuberculous adenitis are benefited or cured entirely when the treatment is watchfully carried out.

It has been a routine procedure in the Connecticut State Sanatoria for very nearly ten years, and has in that time become established as one of the most dependable parts of the treatment plan of these institutions. No effort has been made to supplement or replace natural sunlight with the so called artificial sunlight generated by any of the numerous lamps or lights. The results obtained with natural sunlight have, with few excluded instances, shown the need of no such aid.

PUNCTURE AND INJECTION, OR THE CALOT TREATMENT

Desiring to become personally familiar with certain of the other plans of treatment, and to a certain extent to compare the results obtainable with those which we have become accustomed to expect at the Meriden Sanatorium, we have recently undertaken the study of a plan having the hearty endorsement of Calot (11) in his book, *Indispensable Orthopedics*, published in 1922. The discussion of this method is for that reason somewhat more in detail than those already mentioned. Calot believes that puncture and aspiration of tuberculous lymph nodes, followed by modifying injections, is the simplest, easiest and most dependable treatment for the condition.

The technique of "puncture and injections" which he so favorably commends is briefly as follows:

> Softened lymph nodes, giving evidence of containing fluid, are punctured with a long needle attached to an aspirating syringe. The point of entry is through the healthy skin at some distance from the overlying integument of the diseased lymph node. After the fluid contents of the abscess are withdrawn there is injected into the gland a liquid consisting of olive oil 70 parts, ether 30 parts, creosote 5 parts, guaiacol 1 part, and idioform 10 parts. The quantity of this injected modifying fluid is equal to one-half the amount of pus withdrawn. If the lymph nodes are not softened, but are sclerotic and hardened, liquefaction is induced by the injection of a modifying fluid composed of camphorated naphthol, 2 parts, and glycerine, 12 parts. These injections are made every several days until softening occurs, and then the procedure first described is continued.
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> Punctures and injections are made every 10 days until there is no longer any pus withdrawn. The lymph node, which has been correspondingly diminished in size by the removal of the pus, is aided in its shrinking by having placed over it compression pads and bandages. This assists in coaptating the walls of the abscess and promotes their adherence.

Usually, after the tenth or twelfth puncture and injection, only the faint outline of the lymph node remains barely perceptible and palpable, and the pressure soon completes the cure. Of course, this plan of treatment is not new. Keen (12) tells us that the French surgeon, Verneuil, introduced the method prior to 1890. He removed the tuberculous fluid by means of an aspirator, and then introduced a solution of iodoform and ether. This method was found not to be altogether safe on account of the destructive action upon the tissues of the modifying fluid. Billroth, Krause, the elder Senn, and Callender recommended various modifications (13) or other substances, and Calot himself states that there have been an infinity of medicated agents suggested and, while none is infallible, there are four or five at least which are good, and with which it is possible to obtain a cure provided they are used properly. He asserts that the technique is of a great deal more importance than the nature of the substances injected.

Certain aspects of the plan induced more than casual interest, and one of the angles which invited our particular attention was the statement by Calot as to the method of cure. He asserted that it had been shown by Robin, Coyon, Feissinger and Lawrence that the modifying fluids (14) do not act as antiseptics, but that they act by "provoking a great afflux of white cells, polynuclear cells, afterward destroying them, thus setting at liberty certain ferments, the first a lipolytic ferment having the property of attacking the fatty envelope of the bacillus, later on a proteolytic ferment having the property of liquefying and digesting albuminoids, in other words, a faculty for destroying the very substance of Koch's bacillus."

Keen describes the action in terms somewhat more general:

> It is well known that iodoform retards the growth of the bacillus of tuberculosis, and that its efficiency depends largely upon its acting in a region from which air has been excluded. The inhibitory action on the growth of the organisms when air is excluded is extremely powerful and by the retention of the iodoform in a closed cavity the drug is disseminated over the entire abscess wall, and its action greatly prolonged.

From these two statements it would appear that the method or means by which these substances produced cures was of little interest. Calot's simple unembellished statement carries with it an inference too pregnant with possibilities for us to calmly ignore, however; for the substance which will stimulate the body forces to destroy tubercle bacilli, or will

of itself destroy the bacteria *in vivo* without doing hurt to the tissues, is the substance for which we are all seeking and the substance for which countless investigators have sought and are still seeking.

We are the possessors of more or less accurate knowledge concerning many chemicals and other substances that will undoubtedly destroy the germ *in vitro*, but we are hesitant about transferring their activities to the body. We are more or less familiar with certain assertions and proofs that tubercle bacilli may be made to change their form or even be destroyed, *in vitro*, by modification of environment and pabulary material, but the effect of such procedure upon the bacteria in the body has not been reported upon so fully or so favorably.

In fact, experimentation along these lines has convinced the majority that the drug or substance which is to combat tubercle bacilli in the body must be able to penetrate the tubercle, the cells, if the bacilli are enclosed within cells, and finally the tubercle bacillus, which is apparently one of the most difficult of microörganisms to penetrate.

Lydia DeWitt (15) informs us that

> the action of such a drug or substance may be indirect, increasing or diminishing phagocytosis, or increasing the power of the phagocytes to destroy the bacteria which they have taken up. This might be accomplished by changing the reaction of these cells, making them more acid or more alkaline. As tubercles are usually surrounded by lymphocytes and as lymphocytes have been said by Bergel and others to have a lipolytic power, a drug or substance which would increase the number of lymphocytes or increase their lipolytic power might be imagined to influence the tuberculous condition favorably by breaking down the fatty substances of the tubercle bacilli and thus exposing them to readier destruction by the hostile substance or products of the hostile invasion.

This is much in accord with Calot's belief that the materials that actually accomplish the destruction of the tubercle bacilli are derived from the leucocytes. In order that we may after a fashion visualize this process of disintegration, let us turn again to the series of events which occur when the bacterium enters the body.

We have already stated that when a tubercle bacillus gains entrance into a susceptible host, it immediately becomes the prey of a phagocytic polynuclear leucocyte. But, according to Calmette (16), "The leucocyte lacks the power to destroy the bacillus because the latter is so well protected against the digestive action of the cellular juices by its content in chitin,

waxes and fats." If the bacillus be living and virulent it multiplies in the phagocytes, secretes its poisons and kills the cell. The destruction of the cell sets free in the body fluids, in addition to the mass of young bacilli developed in its protoplasm, debris of degenerated cell nuclei and complex enzymes of leucocytic origin. These enzymes are protease, lipase, amylase, oxydase, complement, etc., and these substances have been the subjects of painstaking and prolonged investigations for a long time, and they are the enzymes which Calot designates as having bactericidal power against the tubercle bacillus. Indeed, before the individual substances had been recognized, their existence had been proved, and efforts were made to utilize them in the treatment of tuberculosis. Rodet (17), Rimbaud (18), Baldwin and Price (19), prior to 1904, macerated tuberculous lymph nodes, made an extract of them, and injected them into tuberculous animals, endeavoring to secure their action against the disease. Various investigators have centered their attention on different products of this contact between leucocyte and tubercle bacillus, and not a few have reported the destructive action of the ferments mentioned upon the microörganism.

What is now known concerning the action and powers of these enzymes may be briefly stated. Müller, Fischer, Abderhalden, Opie and Barker (20) have shown that polynuclear leucocytes of man and other mammals contain an enzyme which digests albuminoid substances in a weakly alkaline medium, and to it has been given the name of protease.

Bergel (21) has demonstrated that lymphocytes contain enzymes which have the property of breaking down fatty substances into fatty acids and glycerine. These enzymes are called lipases and according to Feissinger and Marie (22) they exert a characteristic action upon the tubercle bacillus, evidenced by causing the bacterial elements to become more slender and develop granules after treatment with a fluid containing lipase for 24 hours at 52°C.

It is probable that lipase and protease are the substances referred to by Calot, respectively, as "a lipolytic ferment, having the property of attacking the fatty envelope of the bacillus" and "a proteolytic ferment having the property of liquefying and digesting albuminoids."

The chemicals and drugs which are included in the modifying liquid of Calot are by no means the only substances which have had claimed for them the property of stimulating phagocytosis of tubercle bacilli, or of contributing in other ways to the destruction of the microörganisms in the body.

Various body exudates, other bacteria, many hypothetical ferments obtained from diverse sources, chemicals and chemical compounds galore, have likewise been mentioned as efficient in combating the bacillus *in vivo*, either by phagocytic stimulation, direct bactericidal process, coagulation, agglutination or lytic action.

Thymol, formaldehyde, anilin dyes, salts of copper, gold and bismuth, rare earths of the cerium group, radioactive substances, arsenical compounds, benzyl alcohol, xylol, and any number of other materials, and, since its favorable action upon leprosy was obtained, chaulmoogra oil and hydnocarpic acid have likewise been employed against the tubercle bacillus. Leonard Rogers (23) has written that the fatty acids of chaulmoogra oil, and also those of codliver oil, linseed oil, sardine oil, and soja oil possess the property of altering the waxy capsule of acid-fast bacilli. Most of the experiments referred to have remained unsuccessfully completed. Some few substances there have been for which it has been claimed that action is certain and unvarying, but Calmette has checked many, and has, in the majority of instances, failed to corroborate the findings of their exponents.

To certain drugs is attributed a dynamogenic action, which consists in a stimulation of proliferation of surrounding cells, causing them to circumscribe the tubercle, shutting the bacilli off from oxygen and food, and allowing their own acid and waste products to check their growth and eventually destroy them. Among such drugs are two included by Calot in his so called modifying fluid. These are creosote and guaiacol.

Another of the included drugs is iodoform, and it is said by certain authorities that it produces increased lymphocytosis and in this way increases the lipolytic ferment already mentioned. Ether is included in the medication, perhaps because of its reputed ability as a fat solvent, and also to aid in stimulating the concentration of leucocytes in the area under treatment.

Olive oil, which is the vehicle for the guaiacol, creosote, ether and iodoform, was probably used because of its bland nature, and also because it is one of those oils supposed to act as a direct chemotherapeutic agent, it being denied by certain investigators that it has the property of altering the characteristics of tubercle bacilli by stimulating phagocytosis.

It would appear therefore, theoretically at least, that the modifying liquid of Calot contains substances which might be expected, in view of their reputed powers, to exert a devitalizing effect, if not an entirely destructive action, upon tubercle bacilli *in vivo*, either by direct or in-

direct bactericidal processes, by dynamogenic activity, by stimulating phagocytosis, or by all of these methods in conjunction. And, further, it would seem reasonable to expect that these activities could be more readily promoted and set to work in the lymphoid structure of a diseased lymph node than elsewhere, both because of the ease of direct application and because of the peculiar physiological attributes of the lymph node which have to do with the white-cell elements of blood. In any event, the subject offers an inviting field for research both from a clinical and from a bacteriological point of view.

SUMMARY

As has already been stated, heliotherapy is one important part of the routine procedure at the Meriden Sanatorium, where tuberculous children, exclusively, are treated. This therapeutic measure is applied in all forms of tuberculosis except the moderately advanced and progressively active pulmonary types. The results obtained are, in the main, consistently good, and are responsible for our belief that the plan is probably more efficient than any other single line of therapy.

We have, however, during the past two years had opportunity to observe the Calot method and its effect upon tuberculous lymph nodes. Practically all of the cases that received this form of treatment were at the same time undergoing general heliotherapy; that is, regular exposure of the entire body to the sun, without concentrated effort to expose the diseased areas alone. We have as yet seen no case that the Calot method failed to relieve, and in most instances subsidence of the process has been the result.

Softened and liquefied lymph nodes have responded more promptly and satisfactorily than any other type, and therefore in cases that were to receive this treatment effort was made to induce softening by exposure to the sun. This in most instances produced the results desired, and the aspirations and injections then proceeded at 10-day intervals for an average of six applications. Usually at this time not only had all pus been withdrawn, but the lymph node had been so reduced in size as to be impalpable or only barely palpable.

Smears were made from the pus at each aspiration, and usually little difficulty was experienced in finding tubercle bacilli. The number of leucocytes usually observed in the material of the first aspiration was not as great as might have been expected in tuberculous pus. The pus withdrawn ten, twenty, thirty and forty days after introduction of the Calot

fluid revealed remarkable increases in the number of white blood cells. The fields became more and more filled with products of bacterial digestion and leucocytic disintegration. Phagocytosis was seen in a large number of cells, and morphologically perfect acid-fast bacteria became increasingly rare, and these could not be found in the specimens removed on the thirtieth and fortieth days after the beginning of the Calot injections.

In several of the cases the softened lymph nodes broke down, and the thinned overlying skin opened sufficiently to allow oozing. These openings were sealed with collodion, and extensive destruction of the tissue was avoided by reaspirating. The lymph nodes that did not break down healed without scars, and even those that had drained slightly through the skin, when healed, left behind insignificant and barely noticeable evidence of scarring.

Although the results obtained with the Calot method were consistently favorable, we are not inclined to ascribe too much importance to the part of the plan which calls for the injection of modifying fluids after aspiration.

To prevent breaking down and resulting scarring, to promote healing and to relieve skin pressure, we are in the habit of often aspirating lymph nodes that have become softened by exposure to the sun or otherwise. It has been our observation that cases so handled heal just as quickly, and with just as much permanence and with just as good cosmetic results, as did the cases treated by injecting the modifying materials mentioned. And, likewise, lymph nodes that have become softened, and yet not so liquefied as to demand aspiration, are frequently observed to heal without scarring, simply by the application of the sun's rays in conjunction with the usual routine of the sanatorium.

CONCLUSIONS

Therefore, in the light of what has been said concerning the five plans of treatment under discussion we believe that we may conclude as follows:

1. Tuberculin occupies but little importance in our list of therapeutic aids for tuberculous adenitis.

2. Surgery in competent and conservative hands is applicable to a small percentage of cases, after standard nonsurgical procedures have failed, and in cases in which the removal of lymph nodes is of more importance than the possible development of a paralysis.

3. X-ray treatment has not yet been established on a sufficiently definite basis to warrant conclusions, but reports would seem to indicate bright and favorable prospects, and further reports of its use by unbiased observers should render informative and interesting data.

4. Heliotherapy is undoubtedly successful in promoting more cures of tuberculous adenitis than can ordinarily be anticipated by other means, and this according to Fishberg is a proper gauge for the efficiency of any remedial measure.

5. The Calot method of treatment by aspiration and injection is, in our opinion, highly successful when used in conjunction with general heliotherapy. Whether it is any more successful than simple aspiration, practised in conjunction with heliotherapy, is doubtful, and has not been proved in our experience. We are unable to estimate its efficiency when applied without the aid of suncure.

Finally it should be borne in mind that the proper treatment of tuberculous adenitis rests upon three points, namely:

1. Alleviation of causative or underlying processes, such as treatment of infecting foci in the tonsils, adenoids or teeth.

2. The establishment of good hygiene, with particular attention to good food, fresh air, rest and exercise.

3. The application of a specific remedial agent, the most satisfactory at the present time, in our opinion, being heliotherapy, which may in certain instances be aided by aspiration and in even fewer instances by surgery.

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A TUBERCULOSIS SURVEY OF EXTREMELY UNDERWEIGHT CHILDREN

KATHARINE H. K. WOLFE[1,2]

Authorities are agreed that the majority of tuberculous adults contract their infection before they reach their sixteenth year. Nearly all isolated cases of tuberculosis found among the school children of Lincoln, Nebraska, during the last seven years have belonged to that group of underweight children who are overactive nervously. These two facts have pointed definitely to the importance of locating tuberculosis cases among children, beginning with the extremely underweight group, and providing them with conditions which will cure them. The logical place in which to uncover hidden cases of this disease in children is in the school, as only there can practically all children be reached.

In the fall of 1923 an opportunity to make a tuberculosis survey of underweight children was offered the Board of Education by the Tuberculosis Committee of Lincoln. In answering a questionnaire relating to tuberculosis in school children, which the writer received from this Committee, the statement was added, that if an assistant nurse could be obtained for the School Health Department, it was the writer's personal opinion that most cases of tuberculosis in children would be found in those who are chronically underweight. In answer to this request the Tuberculosis Committee voted to pay the salary of an experienced school nurse for the remainder of the school year (eight months) to assist in a tuberculosis survey of underweight school children. The Board of Education accepted this offer of financial assistance, with the understanding that the survey would be a school project carried out by the School Health Department.

The aim of the Survey was not confined to locating hidden cases of tuberculosis among malnourished children, but also included a study of the actual health conditions of undernourished children and the relation of such conditions to tuberculosis. In other words, the object of the survey was twofold: that of finding existing cases of tuberculosis, and

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that of gaining information which would help further a programme of tuberculosis prevention.

THE SURVEY

Data on the following five items were secured:

1: The Temperature and Pulse Record: The morning and afternoon temperatures and pulse-rate of each child examined, for a school week.

2: The Personal Health Record: The child's disease history, physical defects, clinical symptoms, and living habits having a bearing on overfatigue and a deficiency diet.

3: The Chest Examination Record: All important points obtained from a physical examination of the chest and its organs, the diagnosis of each case, and the special recommendations made by the medical examiner of the chest.

4: The Report to Parents: All abnormal health conditions found in the child, with definite recommendations to meet the need of each condition.

5: The Report to the Family Physician: A definite enumeration of all abnormal chest and other health conditions of the child, the diagnosis of the case, special recommendations, and the names of chest examiner and roentgenologist.

The personnel comprised the following, who were responsible for the study in its entirety:

The School Nurse: She took temperatures and pulse-rates of each child examined, recorded for all examiners, and checked up the specific requirements prescribed for each child in school and home, and in the offices of physicians and roentgenologists.

The Medical Director of School Health: She planned, outlined and supervised the survey, with the help of individual conferences with assisting and other physicians. The Director personally checked the names of all children for chest examination whose temperatures and pulse-rates were indicative of tuberculosis, examined each child as to factors covered on the *Personal Health Record*, examined the completed examination record of each child, and filled out the reports with diagnosis and recommendations for parents and family physicians.

The Three Medical Chest Examiners: They followed a uniform method which covered all important points in chest-examination work. They used a simple code for recording, which made it possible for them to express their findings in uniform terms.

The Roentgenologists: They were physicians of wide experience in their line of work.

All examinations were given to the children without cost to parents. The budget of the School Health Department covered the cost of special chest-examination work, and interested organizations and individuals met the cost of roentgenograms.

The word "tuberculosis" was not used in connection with the survey, in order to avoid possible opposition to the work. The reason given for the survey was "to find hidden causes of underweight."

At the beginning of the survey all children 20 per cent or more underweight in a school building were examined. This plan was soon changed to include all children who were 15 per cent or more underweight when it was found that the number of children 20 per cent or more underweight in a building was too small for a working unit. The total number of children examined in this group was 146.

The work of the survey was started in one of the smaller buildings. The examination record of this group of children was not as complete as that of children examined later, as two children failed to get the heart examination, and a few failed to have their cervical lymph nodes and palms examined.

The children were examined in their own school building during school hours. Groups of ten to twelve children had their temperatures and pulse-rates taken at the same time. Temperatures and pulse-rates were taken of each group twice each day (A.M. and P.M.) at the same hour for a school week. The temperature was taken by mouth with a time allowance of ten minutes. The examination covered on the *Personal Health Record* was given to the children individually and privately. The children were asked concrete questions on the food consumed and living habits practised during the previous twenty-four hours. The facts that the children were acquainted with the Examining Director and assisting nurse, and that the atmosphere was cheerful and the examination informal, caused most of them to volunteer information as to their personal habits spontaneously. It was noted that when children were inclined to falsify in their answers to questions pertaining to personal habits, it was usually to cover up bad habits. Hence, one is justified in assuming that bad habits were even more prevalent than the report indicates.

All children who were recommended for this service and had the consent of their parents were given a chest examination (undressed to below the waist-line). This number comprised 105 children out of the 146

of the *experimental* group and 29 children in the *less underweight* group. Roentgenograms of the chest and sinuses were taken of all children in whom physical examination of the chest gave evidence of tuberculosis or organic heart defects, and who had the consent of their parents.

The percentages given in the tables are based upon the actual number of children for whom reliable information on diseases, defects, clinical symptoms and living habits could be obtained.

THE RESULTS

1. A Study of the Total Number of Children 15 Per Cent or More Underweight: The total number of children examined in the group 15 per cent or more underweight was 146. Of this number 105 were given chest examinations, 19 were not recommended, and the parents of 22 children refused to give their consent for this service. The total number of children (146) had their temperatures and pulse-rates taken for a school week, and were examined on factors covered in the *Personal Health Record.* The results of the completed examination work were tabulated, as shown in tables and graphs 1 to 8.

The specific *Living Habits* under consideration were obtained in order to determine to what extent extremely underweight children practise habits which cause overfatigue, and cause them to eat food which provides them with a deficiency diet (deficient in minerals and vitamines essential for normal growth and development of a child). These two factors are known to be related to reduced alkaline reserve, a lowered resistance to infection and a general low vitality of the body.

The specific *Diseases, Defects,* and *Clinical Symptoms* were obtained in order to gain further information on health conditions associated with tuberculosis, heart defects and focal infections in malnourished children.

Table 1 and Graph 1

These present the percentages of extremely underweight children who practised the enumerated living habits.

The outstanding feature in graph 1 is that an almost perfect negative correlation is found to exist between the consumption of excessive sweets and the consumption of green-leaf and other vegetables except potatoes. The children who consume excessive sweets have no appetite for vegetables. Those children were considered as eating an excessive amount of

TABLE 1

Living habits

Showing the percentage of children 15 per cent or more underweight who practise the listed habits

HABITS	NUMBER OF CHILDREN*	PERCENTAGE PRACTISING HABIT
Sweets, eat excessive amount	137	72.3
Sleep, less than 10 hours	133	60.1
Sleep, less than 9½ hours	133	44.4
Sleep, less than 9 hours	133	23.3
White bread, eat excessive amount	132	57.6
Milk, drink 1 cup or none daily	141	51.1
Milk, drink none	141	32.6
Coffee, drink 1 cup or more daily	141	41.8
Meat, eat excessive amount	137	34.4
Shows, attend nights	133	29.3
Eat vegetables, besides potatoes	140	25.7

* "Number of children" represents those from whom reliable information could be obtained as to their personal habits. The percentage is based on this number.

Note: The total number of children examined was 146; 105 of these had chest examinations; 22 children's parents refused consent for this service; 19 children were not recommended for this examination.

TABLE 2

Diseases, defects and clinical symptoms

Showing the percentage of children 15 per cent or more underweight who have the listed abnormal health conditions

DISEASES, DEFECTS AND CLINICAL SYMPTOMS	NUMBER OF CHILDREN	PERCENTAGE HAVING DISEASES, DEFECTS OR CLINICAL SYMPTOMS
Palms, moist	139	82.0
Cervical lymph nodes, anterior, enlarged	140	82.1
Cervical lymph nodes, posterior, enlarged	137	61.3
Colds, frequent attacks	129	64.3
Tuberculosis, active or probably active	105	60.0
Tonsils, "cryptic"	146	53.4
Cough, with colds, and persistent	128	39.1
Nasal obstruction	146	39.0
Teeth, decayed	146	44.5
Heart, defective	103	43.7
Constipation	135	37.0
Indigestion, frequent attacks	140	34.3
Sinuses, infected	62	29.0
Pneumonia, had attack	126	24.6
Heart, organic defect	103	7.8

Note: This group of children is the same considered in table 1.

sweets who had a strong craving for sweets, those who added an excessive amount of sugar to their food and ate concentrated sweets, such as candy, jelly or jam freely (usually between meals as well as at mealtime).

Nearly 58 per cent ate an excessive amount of white bread. Children were considered as eating an excessive amount of white bread who ate three or more slices of bread at each meal, rarely or never made of unrefined flour. More than half of the children drank little or no milk ("little" refers to one cup of milk daily), and nearly a third of the children drank no milk. More than a third ate a large helping of meat once or oftener each day.

In addition to the high percentages of children who practise the habits of taking insufficient sleep and attending night shows, which bear directly on overfatigue, practically all these delicate children carried the full school programme and extracurricular activities of sturdy children. It was further found that 42 per cent stimulated their already overstimulated nervous system with one or more cups of coffee daily.

Table 2 and Graph 2

These show the percentages of children who suffer from the enumerated diseases, defects and clinical symptoms.

That these children suffer from low resistance to infection appears to be amply verified by the high percentage who suffer from frequent attacks of coryza, "cryptic" tonsils, infected sinuses, tuberculosis and organic heart defects.

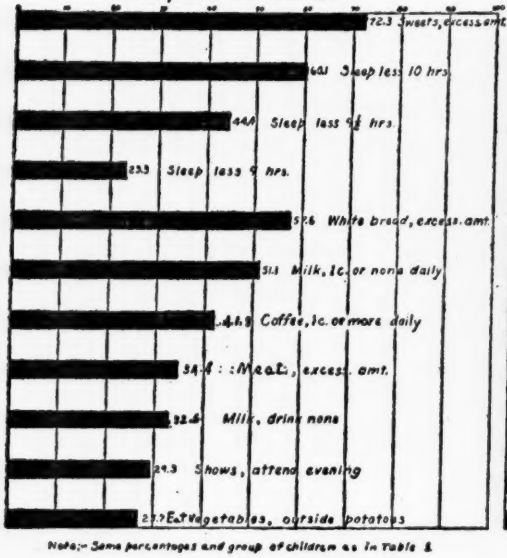
The strikingly high percentage of children found to be afflicted with tuberculosis, in an active or very probably active form, would verify the belief that there are many hidden cases of tuberculosis in extremely underweight children. Most of the cases diagnosed as tuberculosis were found to be in the two earlier curative stages of the disease. All these children had a high pulse-rate, out of proportion to the temperature, and all had a rise of temperature which usually occurred daily. The high percentage of children suffering from moist palms, enlarged cervical lymph nodes, functional heart defects, digestive derangement and constipation would indicate a general low vitality in these children, associated with inefficient functioning of vital parts of the body.

It is believed that a cause-and-effect relationship exists between the high percentage of children, in our group, who practise radically wrong living habits, and the high percentage who suffer from the enumerated diseases, defects and clinical symptoms.

GRAPH 1

LIVING HABITS

Showing the percentage of children 15% or more underweight who practice the listed habits

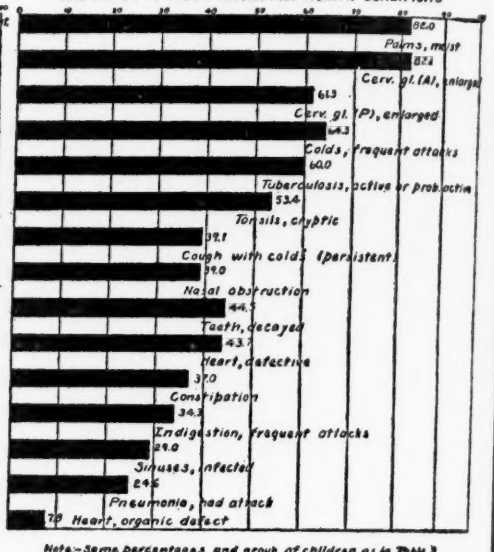


Note:- Same percentages and group of children as in Table 2.

GRAPH 2

DISEASES, DEFECTS AND CLINICAL SYMPTOMS

Showing the percentage of children 15% or more underweight who have the listed abnormal health conditions



Note:- Same percentages and group of children as in Table 3

GRAPH 3

LIVING HABITS

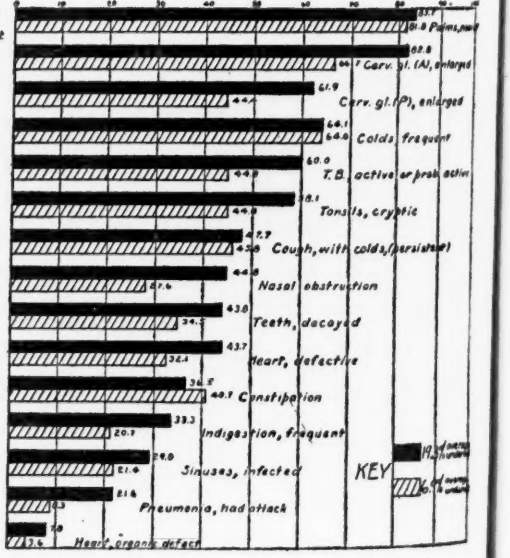
Showing the relation of degrees of bad habits to degrees of malnutrition

Note:- Same percentages and groups of children as in Table 3.

GRAPH 4

DISEASES, DEFECTS AND CLINICAL SYMPTOMS

Showing the relation of degrees of ill-health conditions to degrees of malnutrition



Note:- Same percentages and groups of children as in Table 4.

2. Comparing the Experimental Group with a Group Less Underweight: It is regretted that circumstances did not permit making the same study of a control group of normal children, ranging from average weight to 10 per cent overweight, for comparison. The less underweight group was made up of 29 "special health problem" children in nutrition and high-school gymnasium classes. They ranged 14 per cent or less underweight (average underweight 6.9 per cent), while the experimental group consisted of the 105 children who had chest examination, who ranged 15 per cent or more underweight (average underweight 19.3 per cent). The health examinations of the two groups were the same.

Table 3 and Graph 3

These present a comparative study of the percentages of children practising the listed living habits in the extremely underweight experimental group and in the less underweight group. The use of the less underweight group for the purpose of comparison was not considered until tabulated results of the health examinations revealed interesting differences between the two groups. Could one not infer that these differences, which favor the less underweight group, would very probably have been even greater if the less underweight group had been unselected instead of being special health-problem children?

In considering the habits which cause overfatigue we find that the extremely underweight group indulges the habits of insufficient sleep and attending night shows more than the less underweight group. In comparing the habits of the two groups with regard to a deficiency diet we also find more favorable food habits for the less underweight group of children. This is shown by the higher percentage of children in the extremely underweight group who eat excessively of sweets and a correspondingly small percentage who eat vegetables and the higher percentage who drink no milk. An exception occurs in the case of excessive use of white bread. Here the less underweight subjects indulge this bad habit more than their more underweight companions.

The two groups are found to be practically the same in their habit of drinking little or no milk, drinking coffee, and in eating excessive amounts of meat.

Table 4 and Graph 4

These contain a comparative study of the percentages of the two groups of children considered in graph 3, who suffer from the diseases, defects, and clinical symptoms under consideration.

TABLE 3

Living habits

Showing the relation of degrees of bad habits to degrees of malnutrition

HABITS	15 PER CENT OR MORE UNDERWEIGHT		14 PER CENT OR LESS UNDERWEIGHT	
	Number of children	Percentage practising habits	Number of children	Percentage practising habits
Sweets, eat excessive amount	98	72.4	28	64.3
Sleep, less than 10 hours	94	64.9	27	59.3
Sleep, less than 9½ hours	94	47.9	27	22.2
Sleep, less than 9 hours	94	25.5	27	11.1
White bread, eat excessive amount	93	58.0	28	64.3
Milk, drink 1 cup or none daily	102	49.0	28	50.0
Milk, drink none	102	32.3	28	25.0
Coffee, drink 1 cup or more daily	100	40.0	29	41.4
Meat, eat excessive amount	96	34.4	29	34.5
Shows, attend nights	92	28.3	26	23.1
Eat vegetables, besides potatoes	101	26.7	28	32.1

Note: Total number of children 15 per cent or more underweight 105, average underweight 19. 3 per cent.

Total number of children 14 per cent or less underweight 29, average underweight 6.9 per cent.

Both groups of children had the same health examination under identical conditions.

TABLE 4

Diseases, defects and clinical symptoms

Showing the relation of degree of ill-health conditions to degrees of malnutrition

DISEASES, DEFECTS AND CLINICAL SYMPTOMS	15 PER CENT OR MORE UNDERWEIGHT		14 PER CENT OR LESS UNDERWEIGHT	
	Number of children	Percentage had defects	Number of children	Percentage had defects
Palms, moist	98	83.7	22	81.8
Cervical lymph nodes, anterior, enlarged	101	82.2	27	66.7
Cervical lymph nodes, posterior, enlarged	98	61.9	27	44.4
Colds, frequent	89	64.1	25	64.0
Tuberculosis, active or probably active	105	60.0	29	44.8
Tonsils, "cryptic"	105	58.1	29	44.8
Cough, with colds	88	47.7	24	45.8
Nasal obstruction	105	44.8	29	27.6
Teeth, decayed	105	43.8	29	34.5
Heart, defective	103	43.7	28	32.1
Constipation	96	36.5	27	40.7
Indigestion, frequent	99	33.3	29	20.7
Sinuses, infected	62	29.0	14	21.4
Pneumonia, had attack	88	21.6	24	8.3
Heart, organic defect	103	7.8	28	3.6

Note: These two groups of children are the same as those considered in table 3.

Here again we find the same extremely underweight group of children, who, of the two groups, showed the greater indifference to the practise of right living habits, also suffering most from abnormal health conditions. This is indicated by the higher percentage of children in the experimental group who suffer from all the enumerated diseases, defects, and clinical symptoms, with the exception of frequent colds and moist palms, in regard to which the percentages of the two groups are practically the same, and constipation, for which a slightly higher percentage is found in the less underweight group.

3. Comparing Elementary, Junior and Senior High-School Children: These three groups of children are divisions of the 105 extremely underweight children in the experimental group who had chest examinations.

Table 5 and Graph 5

These contain the percentages of the elementary, junior and senior high-school children who practise the enumerated living habits.

In comparing these three groups, as to percentage of who practise the habit of taking insufficient sleep, we find a continued increase in the percentage of these children, from the elementary through the junior and into the senior divisions, who sleep less than 10 hours. The same is true for those who sleep less than $9\frac{1}{2}$ hours, and for those who sleep less than 9 hours. The difference in ages of these three groups, without doubt, is a causal factor in this difference. When we compare these three groups as to the extent to which they indulge themselves in late hours, after adopting hours of sleep for each group with their average ages in mind, we obtain enlightening information from our report. We will adopt the most favorable record in our report of "less than 10 hours' sleep" for the youngest children or those in the elementary group, "less than $9\frac{1}{2}$ hours' sleep" as no more objectionable for the next older children in the junior high group, and "less than 9 hours' sleep" as no more objectionable for children of senior high age. With this thought in mind, we find in our report a difference of only 2 per cent between the percentage of children in the elementary group who sleep less than 10 hours (40.5 per cent), and the percentage of senior high children who sleep less than 9 hours (38.5). The junior high group, from this viewpoint, indulges itself in the bad habit of taking insufficient sleep far more than the other two groups of children, as is shown by the fact that 51.3 per cent

sleep less than 9½ hours daily. The junior group has also the highest percentage of children who attend night shows. This would indicate that *overfatigue* is a more decided factor in children of junior high age than it is in those of the elementary and senior high divisions. In addition to this record the same junior high division has also the highest percentage of children in the three groups who are in the habit of taking one or more cups of coffee daily.

An interesting statistical record, relating to underweight conditions in junior high pupils in the fall of 1923, when this study was begun, showed that the junior high pupils had the highest percentage (17.7) of children who were 15 per cent or more underweight in the whole Lincoln school system. The next highest percentage was in the senior high school (13.7) and the lowest was that of elementary school children (7.6). The junior high pupils also had the highest percentage of children 7 per cent or more underweight in the whole school system (47.2), with the exception of the children in a very small outlying school building. The average percentage of children 7 per cent or more underweight for the whole school system was 37.4.

In comparing the percentage of children in these three groups who practise habits related to a deficiency diet, we find a continued increase in the percentage of children who consume excessive sweets, from the elementary through the junior and into the senior high groups, and a corresponding decrease in percentage of children who eat vegetables except potatoes. The same increase in percentage through these three groups is found to exist in those who consume an excessive amount of white bread, drink little or no milk, and in the percentage of children who never drink milk.

Table 6 and Graph 6

These present the comparative percentages of the three groups of children shown in graph 5, who suffer from the diseases, defects and clinical symptoms which are being considered.

In comparing these three groups, as to the percentage of children who suffer from physical conditions related to infection, we find that the junior high children suffer most from frequent colds and organic heart defects. The elementary division suffers most from tuberculosis. The report further shows that resistance to tuberculosis increases with age, as is evidenced by the decreasing percentage of children suffering from tuberculosis from the elementary through the junior and into the senior high

TABLE 5

Living habits

Showing the degree to which bad habits are practised by children in different age groups

HABITS	ELEMENTARY		JUNIOR HIGH		SENIOR HIGH	
	Number of children	Percentage practising habits	Number of children	Percentage practising habits	Number of children	Percentage practising habits
Sweets, eat excessive amount	46	69.6	39	74.4	13	76.9
Sleep, less than 10 hours	42	40.5	39	82.1	13	92.3
Sleep, less than 9½ hours	42	33.3	39	51.3	13	84.6
Sleep, less than 9 hours	42	11.9	39	35.9	13	38.5
White bread, eat excessive amount	46	41.3	35	71.4	12	83.3
Milk, drink 1 cup or none daily	49	38.8	40	52.5	13	76.9
Milk, drink none	49	24.5	40	35.0	13	53.8
Coffee, drink 1 cup or more daily	47	31.9	40	47.5	13	46.2
Meat, eat excessive amount	45	33.3	38	36.8	13	30.8
Shows, attend nights	44	13.6	38	42.1	10	40.0
Eat vegetables, besides potatoes	47	29.8	41	26.8	13	15.4

Note: These three groups comprise the 105 children (15 per cent or more underweight) who had the complete health examination, including the chest.

The total number of children in each group is elementary 51, junior high 41, and senior high 13.

TABLE 6

Diseases, defects and clinical symptoms

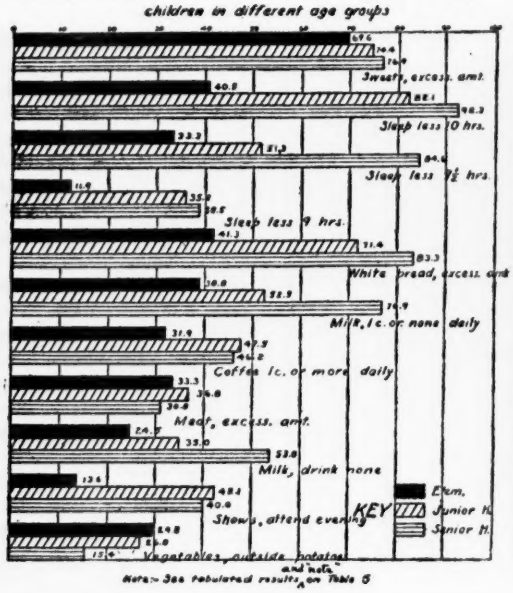
Showing the degree of ill-health conditions found in children in different age groups

DISEASES, DEFECTS AND CLINICAL SYMPTOMS	ELEMENTARY		JUNIOR HIGH		SENIOR HIGH	
	Number of children	Percentage had defects	Number of children	Percentage had defects	Number of children	Percentage had defects
Palms, moist	47	80.9	38	89.5	13	76.9
Cervical lymph nodes, anterior, enlarged	50	94.0	38	76.3	13	53.8
Cervical lymph nodes, posterior, enlarged	47	63.8	38	65.8	13	38.5
Colds, frequent	40	62.5	37	70.3	12	50.0
Tuberculosis, active or probably active	51	64.7	41	56.1	13	53.8
Tonsils, "cryptic"	51	64.7	41	53.7	13	46.2
Cough, with colds	40	55.0	36	44.4	12	33.3
Nasal obstruction	51	49.0	41	46.3	13	23.1
Teeth, decayed	51	45.1	41	43.9	13	38.5
Heart, defective	50	40.0	41	48.8	12	41.7
Constipation	46	43.5	38	31.6	12	25.0
Indigestion, frequent	46	32.6	40	37.5	13	23.1
Sinuses, infected	31	19.2	22	36.4	9	44.4
Pneumonia, had attack	36	16.7	40	25.0	12	25.0
Heart, organic defect	50	8.0	41	9.8	12	0

Note: These three groups of children are the same three divisions considered in table 5.

GRAPH 5
LIVING HABITS

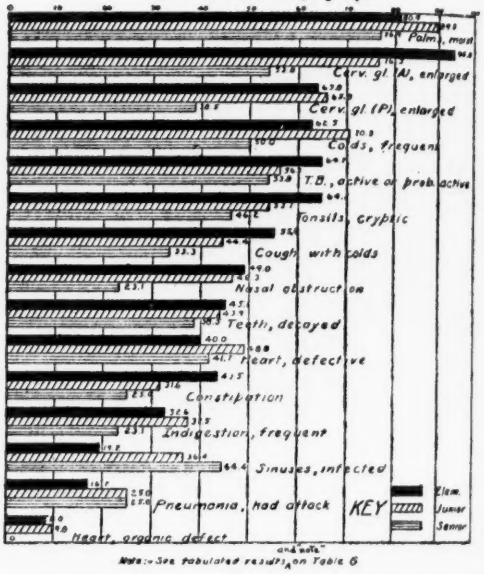
Showing the degree to which bad habits are practiced by children in different age groups



Note:- See tabulated results A and B on Table 5

GRAPH 6
DISEASES, DEFECTS AND CLINICAL SYMPTOMS

Showing the degree of ill-health conditions found in children in different age groups



Note:- See tabulated results A and B on Table 6

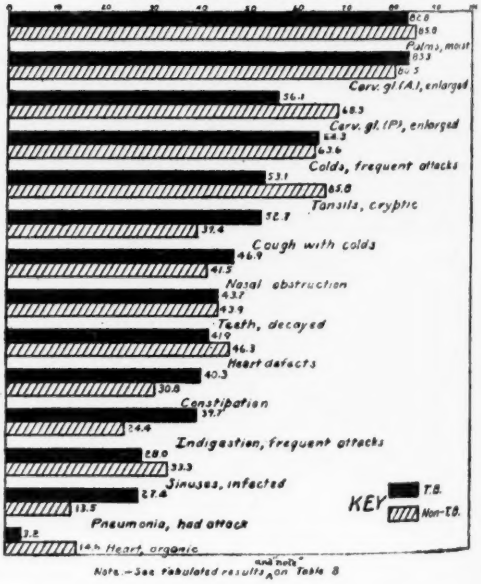
GRAPH 7
LIVING HABITS

Showing the degree to which bad habits are practiced by tuberculous and non-tuberculous children

Note:- See tabulated results A and B on Table 7

GRAPH 8
DISEASES, DEFECTS AND CLINICAL SYMPTOMS

Showing the degrees of ill-health conditions present in tuberculous and non-tuberculous children



Note.—See tabulated results A and B on Table 8

divisions. The reverse condition is found to exist in the case of infected sinuses. Here we find that resistance to this disease condition is lowered with age, as shown by the fact that the senior high division has the highest percentage of children suffering from infected sinuses while the elementary division has the lowest percentage. The elementary group, in addition to having the highest percentage of children who suffer from tuberculosis, also have the highest percentage who are afflicted with "cryptic" tonsils.

In comparing the percentages of children in these three groups who suffer from abnormal health conditions related to a general low vitality of the body, we find that the children in the junior high division, who indulge themselves most in wrong habits which cause overfatigue and drink more coffee than the two other divisions, suffer most from functional heart defects, moist palms, enlarged posterior cervical lymph nodes, and frequent attacks of indigestion. The elementary division, in addition to suffering most from tuberculosis and "cryptic" tonsils, also had the highest percentage who suffer from persistent cough with colds, enlarged anterior cervical lymph nodes, nasal obstruction and constipation.

4. Comparing Tuberculous with Nontuberculous Children: The 105 children in the experimental group who had chest examinations were divided into the group of 64 children found to have tuberculosis, and the group of 41 nontuberculous children.

Table 7 and Graph 7

These contain the comparative percentages of children practising the enumerated living habits.

In comparing the living habits of these two divisions of children, we find a strikingly clear line drawn between the two groups in the practice of habits which lead to overfatigue, and those which are related to a deficiency diet. The tuberculous group of children has a much higher percentage of children than the nontuberculous division, who take insufficient sleep, attend night shows, and stimulate themselves with coffee daily, whereas the nontuberculous group of children has a percentage of children, which is just as decidedly higher than the tuberculous group, who eat an excessive amount of sweets with a corresponding deficiency of vegetables, an excessive amount of white bread and meat, and who drink no milk.

TABLE 7

Living habits

Showing the degree to which bad habits are practised by tuberculous and nontuberculous children

HABITS	TUBERCULOUS CHILDREN		NONTUBERCULOUS CHILDREN	
	Number of children	Percentage practising habits	Number of children	Percentage practising habits
Sweets, eat excessive amount	58	69.0	40	77.5
Sleep, less than 10 hours	56	71.4	38	55.3
Sleep, less than 9½ hours	56	55.4	38	36.8
Sleep, less than 9 hours	56	30.4	38	18.4
White bread, eat excessive amount	55	45.5	38	76.3
Milk, drink 1 cup or none daily	62	45.2	40	55.0
Milk, drink none	62	27.4	40	40.0
Coffee, drink 1 cup or more daily	60	45.0	40	32.5
Meat, eat excessive amount	57	26.3	39	46.2
Shows, attend nights	55	32.7	37	21.6
Eat vegetables, besides potatoes	60	28.3	41	24.4

Note: These two groups comprise the 105 children (15 per cent or more underweight) who had the complete health examination, including the chest.

The total number of children in each group is tuberculous 64, and nontuberculous 41.

TABLE 8

Diseases, defects and clinical symptoms

Showing the degrees of ill-health conditions present in tuberculous and nontuberculous children

DISEASES, DEFECTS AND CLINICAL SYMPTOMS	TUBERCULOUS CHILDREN		NONTUBERCULOUS CHILDREN	
	Number of children	Percentage having defects	Number of children	Percentage having defects
Palms, moist	58	82.8	40	85.0
Cervical lymph nodes, anterior, enlarged	60	83.3	41	80.5
Cervical lymph nodes, posterior, enlarged	57	56.1	41	68.3
Colds, frequent	56	64.3	33	63.6
Tonsils, "cryptic"	64	53.1	41	65.8
Cough with colds	55	52.7	33	39.4
Nasal obstruction	64	46.9	41	41.5
Teeth, decayed	64	43.7	41	43.9
Heart, defective	62	41.9	41	46.3
Constipation	57	40.3	39	30.8
Indigestion, frequent	58	39.7	41	24.4
Sinuses, infected	50	28.0	12	33.3
Pneumonia, had attack	51	27.4	37	13.5
Heart, organic defect	62	3.2	41	14.6

Note: These two groups of children are the same considered in table 7.

Table 8 and Graph 8

These present the comparative percentages of children in the tuberculous and the nontuberculous groups (same groups considered on graph 7) who suffer from the listed diseases, defects and clinical symptoms.

In comparing the percentages of children who suffer from disease conditions related to infection, we find that the nontuberculous division suffers much more from these conditions than does the tuberculous group. This is shown by the higher percentage of children in the nontuberculous group who suffer from "cryptic" tonsils, infected sinuses and organic heart defects.

The tuberculous group has a higher percentage of children who suffer from persistent cough, colds, nasal obstruction, constipation, and frequent attacks of indigestion. The two groups of children suffer to the same extent, practically, from frequent colds, decayed teeth, and enlarged anterior cervical lymph nodes. The tuberculous division has twice as large a percentage of children, who have a disease history of pneumonia as the nontuberculous group of children.

SUMMARY

1. The vast majority of underweight children live on a deficiency diet, consisting chiefly of acid-forming food.

2. Almost a perfect negative correlation exists in extremely underweight children between their consumption of an excessive amount of sweets and loss of appetite for vegetables, with the exception of potatoes.

3. A cause-and-effect relationship seems to exist between the radically wrong living habits practised by underweight children and the high percentage who suffer from the diseases, defects and clinical symptoms considered in this study.

4. A relationship seems to exist between the extent to which underweight children suffer from the enumerated diseases, defects and clinical symptoms, and the degree of malnutrition with which they are afflicted.

5. A relationship seems to exist in these children between their desire to practise habits which cause overfatigue and their desire to drink coffee.

6. Overfatigue and coffee-drinking are more decided health factors in extremely underweight junior high pupils than they are in elementary or senior high extremely underweight children. This group likewise suffers more from heart defects than the other two groups.

7. Extremely underweight children do not naturally outgrow the habit of eating a deficiency diet as they grow older.

8. Extremely underweight children of elementary school age suffer more from diseases and defects of the lymph nodes than those of junior and senior high school age. This is shown by the fact that the elementary division of the experimental group of children has the highest percentage of children who suffer from tuberculosis (usually found in the lymph nodes), "cryptic" tonsils, adenoids and enlarged anterior cervical lymph nodes.

9. Resistance to sinus infection in extremely underweight children grows less as they grow older.

10. Extremely underweight elementary school children suffer more from all listed diseases, defects and clinical symptoms than extremely underweight children in the senior high group, with the exception of heart defects and infected sinuses.

11. Tuberculous children indulge themselves more than the nontuberculous group in habits which cause overfatigue and in the habit of coffee-drinking. This fact points to a probable causal relationship between overfatigue and coffee-drinking and latent tuberculosis becoming active. The tuberculous children suffer more from persistent cough accompanying colds, nasal obstruction, sluggish elimination and gastric derangement than the nontuberculous children.

12. Nontuberculous children indulge themselves more in the practice of food habits which provide them with a deficiency diet than does the tuberculous group of children. They likewise suffer more from infections of tonsils, sinuses, and the heart than does the tuberculous group of children. This fact supports the belief that eating a diet deficient in minerals and vitamines lowers resistance to infections.

13. Tuberculosis is found to be a common disease in extremely underweight children (60 per cent) when sufficient time and care are given to the health examination of these children to locate lymphatic disease (before involvement of the lungs) as well as advanced cases of this disease.

14. Malnutrition seems to lay the foundation, not alone for tuberculosis, but also for other serious disease conditions in children. This indicates the importance of locating the causes for malnutrition in all children who are chronically underweight.

15. It is possible and practical, not alone to locate cases of tuberculosis in malnourished children, but also, with the same health examina-

tion, to find hidden cases of heart defects, focal infections and other underlying causes for their delicate condition of health.

16. The responsibility for the causes of malnutrition found in the children who enter into this study, to be placed where it belongs, would be in the home, the school and the community. A constructive health programme which would remove these causes would demand that the home, the school and community organizations, not alone correct bad health conditions for which they have been directly responsible, but also that they coöperate with each other in furthering a health programme which will wipe out malnutrition, and with it tuberculosis and other preventable and correctable disease conditions of children, from the community. It is hoped by the writer that opportunity may present itself in the near future to follow up this report with an outline for a practical campaign against the causes of tuberculosis found in the present study.

THE CARE OF THE TUBERCULOSIS PREVENTORIUM CHILD

EVERETT K. GEER[1]

The following rather brief remarks are the result of six years' experience as attending physician to the Children's Preventorium of Ramsey County, Minnesota, which accommodates 75 children between the ages of five and twelve years. These remarks have been stimulated chiefly by the dearth in medical literature of material relating to the preventorium, its object, measures utilized, and its results.

Our conception of the preventorium child is one between the ages of five and twelve, who has been intimately exposed to tuberculosis, who gives a positive skin response to tuberculin, and whose general condition is below that which is supposedly normal; briefly, below-par children with tuberculous infection.

Our conception of the object of the preventorium is to restore these children to a normal physical condition if possible, with the hope and growing conviction that by so doing we are helping to diminish our future consumptive population.

The measures we are employing at the Ramsey County Preventorium are: (1) rest in fresh air, (2) supervised activities, (3) a well-balanced and adequate diet, (4) prompt eradication of infectious foci, (5) heliotherapy and air-baths, (6) tuberculin, (7) open-air school-rooms, (8) child guidance survey, and (9) follow-up work.

When a child has passed the entrance examination, it is vaccinated against smallpox, unless it exhibits a scar of recent origin, and its throat is cultured for diphtheria. After admittance to the institution it has been given diphtheria toxin-antitoxin in the past. From now on it is our intention to immunize against diphtheria and scarlet fever with the soap toxins developed by Dr. W. P. Larson of the University of Minnesota.

For the first week after admission, a temperature, pulse and respiration chart is kept. Should there be any fever (a very rare thing) the child is kept in bed; otherwise, it enters at once into the customary routine. There is no time limit of residence, children having stayed as long as five years before we were willing to send them home.

[1]St. Paul, Minnesota.

It is felt that rest in the fresh air is as important for these small patients as for our clinically tuberculous people. All our children sleep on porches with two or three exposures. Twelve hours in bed at night, with two additional hours during the day, is the rule, with additional hours if deemed advisable. During rest-hours quiet reigns supreme; a disinterested relaxation is our object. The benefits to be derived from resting in the *fresh* air are sufficiently well known to all.

Children, of course, turned loose are greatly inclined to run their legs off. Constant and often purposeless activity is almost synonymous with childhood. Knowing the improvidence of children we have deemed it wise and essential to curb somewhat their animal-like restlessness. Hikes, games, swimming, skating parties, etc., are all supervised, and kept well within the limits of their endurance. Exercise to the stage of stimulation, but not to the point of fatigue, is the ideal. Intelligent supervision closely approaches this goal.

Practically all these children come to us below weight; some from lack of sufficient or proper food, others because their tuberculous infection is on the threshold of tuberculous disease, and still others because other infections have taken their toll. Consequently, food of the right kind and in sufficient quantities is an imperative need and one which is fulfilled without stint. Our milk bill is huge, our grocery account still more formidable. With a few children who failed to gain weight satisfactorily, goat's milk has been tried but with no marked success. Cod-liver oil is dispensed freely, for both its food value and vitamine content.

All of our children come from poor families; so it is to be expected that their mouths and throats will need attention, and we are seldom disappointed. It is remarkable, in many instances, to note the quick improvement after carious teeth have been removed or filled and bad tonsils taken out. The Wilder Dental Clinic in St. Paul is contributing no small amount to the success we have in rebuilding these children.

Sun-baths are given routinely, beginning early in the spring and according to the Rollier technique. During the summer, short trunks are the only clothes worn. When the cool weather in the fall precludes exposure to the sun, air-baths are given which continue throughout the winter. Since giving the sun-baths and following through the winter with air-baths, the incidence of upper respiratory infections has been very low. Until recently we have had no electricity at the Preventorium, which has prevented the use of the ultraviolet lamp in the winter, but a stable current is now available and a lamp room is being constructed,

so that by January we will be equipped to continue light therapy during the winter months.

Tuberculin occupies a rational place in the care of the preventorium child. Sir Robert Philip, before the Tuberculosis Society of Scotland in Edinburgh in February, 1923, made a strong plea for tuberculin, claiming that "it exerts a remarkable inhibitory influence on the first buddings of tuberculosis in childhood," and asserting that "continued observation as to its effects throughout long periods in many types of tuberculosis has led me to conclude that the method, consistently applied, is capable of effecting the nearest approach to *de*tuberculization yet realized." It certainly must be admitted that tuberculous infection in childhood, as a rule, piles up in lymph nodes, and it must also be agreed that tuberculin is of marked value in treating tuberculous lymph nodes. The conclusion is obvious. We have noted many times that these children will gain more surely and rapidly if given tuberculin, and in a number of children that no gain in weight occurred until tuberculin was given. The fallacy of the "cause-and-effect" argument is fully appreciated, but in more than one glaring instance of failure to gain weight this relationship of tuberculin and gain has been noted, and so strikingly as to arrest our attention. As yet, our routine does not include tuberculin, but we are strongly tempted to make it so.

School work is an important factor in our treatment. Some children stay with us three or four years; so the need of the school-room is imperative, and it is an open-air school room with windows open. No child is in school for more than two hours a day, and yet it is possible to keep up with the city school curriculum.

I hesitate somewhat to speak of the result of the survey and subsequent work done by the Child Guidance Clinic under the direction of Dr. M. L. Stiffler. This work has been going on for only one year, and we do not want to be misunderstood in some of the conclusions we have drawn because we may see fit to change them later. Our problem essentially was twofold: a certain number of children had been with us for several years with no apparent improvement, and the school work was becoming increasingly difficult with several outstanding behavioristic problems. I do not propose to go into the whole problem. The high lights from Doctor Stiffler's investigation were: (1) 28 of 75 children could qualify for admission to feeble-minded schools; (2) in the majority of instances the children who failed to gain physically were those who stood lowest

mentally; and (3) no appreciable mental improvement occurred with physical gain. The school problem was largely solved when we knew the mental material we were dealing with. A great deal was learned regarding the question of behavior, and no little help rendered to remedy the difficulties. The question arose as to whether it was right or wise to admit all children to the Preventorium indiscriminately, without having a mental and behavioristic diagnosis. There is no question that in the past we have been spending considerable time and much money in an attempt to build up persons who will never be able to take their proper place in society. At present we are picking our children, and admitting those whom we think will be community assets instead of liabilities. We feel that it is within our province thus to choose those whom we will admit, and we are asserting that prerogative. Before a child is now admitted to the Preventorium of Ramsey County he must go through the Child Guidance Clinic as well as be examined by the admitting physician. It is recognized that this departure is liable to bring down on our heads a storm of criticism, especially from those whose interest in public-health work and preventive medicine is governed by maudlin sentimentality rather than by cold reason. But we risk this abuse, feeling very strongly that medically and biologically we are right.

Follow-up work is as necessary for the preventorium as for the sanatorium; otherwise, we would not know what we achieve. Such is the industry of our follow-up worker that we know the condition of all but 7 of our 223 children discharged since 1915. We know that but two of them have developed tuberculous disease, and one of these has died, due, we feel, in large measure to the fact that she was dragged into a life of prostitution by her mother shortly after leaving the institution. We know also that the physical condition of the big majority is most satisfactory. Our follow-up worker has the girls enrolled in an alumni association and the boys organized into a Preventorium Booster Club to keep in touch with our graduates.

Of course, from all this the children absorb the rules of health-producing living. The necessity for brushing their teeth, sleeping in fresh air, going to bed early, eating regular meals, keeping their bodies clean, and so forth, becomes an integral part of their thoughts and actions. Consequently, they leave as health crusaders in fact. Many an amusing tale has come to us as to how homes have been improved through the lessons learned by the children while at the Preventorium.

Such, in brief, is our idea of the care the preventorium child should have. It no doubt is far from perfect, but we know from experience that the vast majority of below par children with tuberculous infection will be restored to a physical condition closely approximating the supposed normal by such treatment, and we feel that this restoration to more perfect health is a part of antituberculosis work which cannot logically be disregarded.

CLINICS AND FOLLOW-UP WORK AFTER SANATORIUM TREATMENT FOR TUBERCULOSIS[1]

ARTHUR T. LAIRD[2]

Clinics connected with sanatoria serve several purposes. The first and most obvious one is to afford a diagnostic station to which suspected cases may be referred and where an applicant for admission to the sanatorium may have his condition established and the urgency of his case determined. The second and scarcely less important function of the clinic is its use as a rallying centre for former patients to which they may feel free to come for examination and advice. Education of the patients and their friends is a third function.

The diagnosis of tuberculosis is not such a simple matter that even a skilled physician can hurriedly give a dozen or more persons single cursory examinations and dogmatically pronounce them either tuberculous or not tuberculous. Localized râles at an apex can, to be sure, be detected in a moment in clear-cut cases, but the day is past when a doctor can safely slap a husky-looking "suspect" on the back and assure him, without more than a brief contact with the stethoscope, that he has the finest pair of lungs in the country. Time is an element in the diagnosis, and although time is priceless and must not be lost in the treatment of an active case, it is poor economy not to take sufficient time to establish either a positive or a negative diagnosis. Consequently in the majority of cases it is necessary to see the "suspect" more than once. Our routine in the Duluth dispensaries is as follows:

1. Secure diagnosis, if possible, after first
   a. Chest examination
   b. Sputum examination
   c. X-ray examination
2. If negative on first examination secure before dropping case
   a. Second chest examination
   b. Three sputum examinations
   c. Several afternoon temperatures

[1] Read at the annual meeting of the Minnesota Sanatorium Association, St. Paul, Minnesota, December 17, 1925.

[2] Superintendent, Nopeming Sanatorium, Nopeming, Minnesota.

The history taken at the first visit and the record of the patient's symptoms are of course important factors in the diagnosis.

The clinics in Duluth are held twice a week, the one at St. Luke's Outpatient Department on Tuesday morning, and the other at St. Mary's Hospital Outpatient Department on Saturday morning. These clinics are the clearing houses for the entire county system of clinics, and to them doubtful cases are referred from these outside centres.

The sessions of the clinics in Duluth are attended by members of the sanatorium staff, by the director of social service and the follow-up nurse, and by the tuberculosis nurses of the Duluth Health Department. The stereo X-ray films are made during the clinic session in the hospital X-ray laboratories.

If a patient comes from a distance it may be possible to give him a definite diagnosis the same day, especially if symptoms, and X-ray and chest examinations are all negative. If he is a local resident he is ordinarily asked to return the following week for a second chest examination at which time the reports of sputum and X-ray examinations will be available, together with temperature records.

If, after his second visit to the clinic no definite evidence of tuberculosis is found, the case is diagnosed as not tuberculous, but the patient is asked to report in one month as to the continuance of symptoms.

Ordinarily, the attendance at the Duluth clinics numbers from ten to fifteen at each session. Patients may also be examined at the sanatorium, and occasionally suspects are admitted for a time and kept under observation until the diagnosis is clear.

The outlying clinics are held once a month in each of six cities on the iron ranges, and are conducted either by Dr. H. G. Lampson, the county health officer and formerly a member of the sanatorium staff, or else by one of the physicians from Nopeming. He is assisted by the local city or school nurses. Frequently one or more of the county rural nurses is also present with suspects or contacts to be examined. Ordinarily, a final diagnosis is not made except in obvious cases, but suspects are asked to return again in a month, when, if still negative, they are considered as not tuberculous. If the symptoms indicate the need for a prompter decision the patient is referred to one of the clinics in Duluth or to Nopeming for stereo X-ray examination, although in the large centres flat chest plates are available. In some urgent cases

the patient is admitted to one of the Duluth hospitals or Nopeming for more thorough study.

Follow-up is a term that suggests thoroughness. It is not simply following, going over the same road at a distance, after what you want is gone. It means securing the full benefit of an achievement or enterprise. In war it means securing the fruits of an advantage gained or a victory won. In its broadest sense follow-up may be profitably applied to any department of hospital or sanatorium activity. A number of valuable results may be lost because the follow-up process is not begun early enough. If a patient's name is put on the waiting list, and for some reason he doesn't come, there is an opportunity for follow-up. In fact, the making of the diagnosis affords the best starting point for follow-up. The follow-up department of a sanatorium should ensure permanent results for treatment. This is now well recognized in the general hospital. Hospital social service is inevitably very intimately related to efficient follow-up work, and is a part of it. If conditions are such as are not conducive to the result wanted the conditions may be changed. A dozen different agencies can help, and will help, if enlisted. Unless the work of the sanatorium is followed up and confirmed in the securing of permanent results for individual patients, all the patients are likely to suffer, and much of the activity becomes mechanical routine.

The follow-up of discharged patients should therefore be begun with their discovery. Of course, another sort of following the patients' course may be used which perhaps has some statistical value and indirectly may be of service to subsequent sufferers, but it lacks the live human appeal that real service possesses. Dr. Michael Davis says "finding an epitaph at the end of a year" is not nearly as useful as "forestalling the undertaker."

There are scores of matters which concern the follow-up department from the day of the patient's first visit to the clinic or the arrival of the application from a private physician in the mail.

If there is a long waiting list at the local sanatorium the treatment can sometimes be begun in another institution and possibly be continued there for an indefinite period.

The State Sanatorium is available for minimal and quiescent cases, but these form only a small percentage of our clients, and it is difficult to persuade patients to go so far from home. Moreover, the State Sanatorium may have a waiting list of its own.

Private sanatoria are within reach of the resources of some, especially if it is known that the expense involved will be necessary for only a limited period or until there is an opening at the local institution. If there is not likely to be a vacancy there for a month or two the patient can often be admitted promptly to a private sanatorium.

In Duluth arrangements have now been made so that, pending admission to the sanatorium, patients can be admitted to private rooms or special wards in a general hospital. They can remain there under the supervision of the sanatorium staff and the resident physicians until places can be found for them at the sanatorium. Their expenses there, if they are without resources, may be paid out of the sanatorium fund.

A few St. Louis County patients are placed in other county sanatoria, but this resource, used to a considerable extent in former years, is now less frequently employed, as many of these places have waiting lists of their own.

Only when it is unavoidable, and the patient's home surroundings and conditions are unusually satisfactory, is remaining at home encouraged, even for the period that the patient awaits admission to the sanatorium.

It is not always possible to persuade the patient to go, even when prompt admission to a suitable institution can be secured. The mere refusal to consider the opportunity is practically never to be taken as sufficient reason for the patient's remaining at home without follow-up. The refusal may be due to misunderstanding of the nature of the sanatorium or the treatment, of the expense involved, or the responsibilities of the patient to his family in the prevention of infection. Some of these difficulties may be cleared up in a visit by one of the follow-up workers or nurses. When the patient still declines the privilege offered him, his admission may be sometimes effected by other means. Economic pressure may be brought through the securing of a mother's pension for his wife, provided he remains at the sanatorium, or he may be definitely committed to the sanatorium by the County Commissioners upon complaint of a health officer that he is a menace to his associates. Both of these measures have been employed in St. Louis County during the past year. Their use requires considerable discretion, and the cases should be carefully studied by the follow-up worker before they are recommended.

Once in the sanatorium there are other matters concerning the patient which crowd the follow-up worker's programme. Keeping steadily in mind the patient's prospective return to his family, it is necessary to study the home and the other members of the family more closely, and no better opportunity to become acquainted can be found than at the time of their visits to the patient at the sanatorium. Sometimes it is possible to arrange for their examination during these visits, or they may be asked to attend the clinics. The repeated visits bring closer acquaintance, and, if utilized to full advantage, a considerable store of knowledge of the family characteristics and resources can be obtained.

The physicians at the sanatorium can be aided in making prompt and full study of the case if the data secured in the clinics, hospitals and by private physicians is obtained for use in the medical office. As soon as the preliminary examinations are completed the patient's friends and his physician should be informed of the findings, so that the situation may be clear and the problems arising from it faced squarely. In the securing and forwarding of these reports the follow-up worker should share.

During the patient's stay at the sanatorium many situations arise requiring the services of the follow-up worker. A good deal of her activity for him takes the form of correspondence and telephoning in regard to various emergencies that arise. Many social agencies may have to be consulted, and personal visits made to ensure him the benefit of sanatorium treatment undisturbed by worry about home conditions.

After the Sanatorium—What?

This query given me by your President is an insistent one. After all, what is to become of these patients in whom the community has invested, not only considerable sums of money, but a sympathetic interest and considerable skilful attention? What is to become of these patients who have had X-ray plates, pneumothorax and sun-cure, surgery and occupational therapy? How thoroughly have they been rehabilitated and how long will their reconstructed usefulness last?

Here is one of the special responsibilities of the follow-up department.

Unfortunately, many patients return home so ill that about all the follow-up worker can do is to help prevent infection of other members of the family. If it were possible to keep them all there, such patients should not go home at all but should remain at the sanatorium as long as they live. Since they form the majority of the patients at the insti-

tution many of them do go home whether we like it or not. Sometimes they can be persuaded to return. The real remedy for the situation is earlier diagnosis so that they may be discharged able-bodied and useful.

For those with quiescent and arrested disease constant supervision and sheltered employment must be provided, and an industrial colony for ex-patients would solve some of the employment problems. Patients recently discharged from the sanatorium require more frequent visits than do those whose reaction to the new environment is already established and is somewhat stationary. The clinic offers a convenient centre for meeting ambulant ex-patients and for periodically keeping track of their condition. How often an ex-patient should be seen depends on his condition.

The following outline for the *Control of Patients* was prepared several years ago for the Visiting Nurses of the Duluth Health Department:

I. Positive sputum cases with active symptoms
  1. Get into an institution at once if possible
  2. Keep track of removals
  3. Furnish sputum supplies
  4. Supply literature
  5. Supervise while at home
    a. Get patient at rest and arrange for fresh air as prescribed
    b. See that directions are followed as regards treatment and hygienic precautions
  6. Secure the examination of all the others exposed, at least all the other members of the family
  7. See at dispensary or visit at home every two weeks if practicable (oftener if careless)

II. Positive sputum cases without active symptoms. Cases with positive sputum under dispensary care to be seen at least once a month whether they have symptoms or not. Dispensary positive chest cases with negative sputum (arrested cases) to be seen once in two months. Private cases to be seen once in two months unless physician in charge objects.

III. Positive chest cases with negative sputum, but with active symptoms
  1. To 5 same as for positive sputum cases
  2. See at least once a month

IV. Negative cases in tuberculous families
  1. See at the dispensary if possible once in six months

V. Negative cases in nontuberculous families
  1. When diagnosis has been completed, drop

VI. Other than chest cases (bone, joint, lymph nodes, etc.)
1. Secure institutional care, or supervise

It has not been possible to carry out the above procedures consistently with the staff of workers available, but it seems to us a reasonable goal that should be attained.

In the *Biennial Report of the Chicago Municipal Sanitarium*, just received, under the heading *Dispensary Nursing Service* the statement is made that all pulmonary cases are visited once each month, and more often if necessary. Lymph-node cases are visited once every three months, and more often if necessary. In the same report, under the heading *Dispensaries Medical Service*, it is stated that the Chicago Health Department enforces the rule that no child under sixteen years of age shall be present in the home of a positive-sputum case of tuberculosis. Either the tuberculous individual must leave the home and enter an institution for the treatment of his disease, or separate himself from the children in some other abode, or the children must be removed from the home.

The organization of the work in Chicago is quite elaborate, and more comprehensive than is possible in most communities. The bulletin containing the report is most interesting and can be readily obtained. Our organization in St. Louis County is relatively simple, and consists of the sanatorium staff, the clinics, one field worker, one social service worker, and a clerk. The County Public Health Association has helped to pay the salary of the trained social worker we have had since July first. Her help has been invaluable. Working in close coöperation are the county and local city health departments and about thirty public health nurses.

The work already started in your institutions is doubtless proceeding along similar lines. While the workers may be fewer in number, the principles are the same, and the underlying purpose of the whole enterprise is to follow-up what has been begun, until it is as well done as is possible with the resources we have. Eventually, with whole-time health officers and a sufficient nursing staff, a larger portion of the burden of follow-up may fall upon the health departments than they can assume at the present time. Meanwhile, it seems to be the sanatorium's responsibility, and many health workers believe it should be so permanently.

Discussion by Dr. H. Longstreet Taylor

In Doctor Laird's paper on the after-care of our sanatorium graduates and in Doctor Geer's remarks in regard to the follow-up work among the children discharged from the Preventorium, the need and importance of this service have been emphasized for both classes of patients. Patients from any institutions, in which tuberculosis in any of its myriad forms is cared for, are always in danger of relapse for a long period of time after their discharge. Our follow-up work has its weakest point in the fact that from its very nature it is and has to be intermittent, and not continuous as it should be to be efficacious. To meet this requirement the working colony for ex-institutional patients is the obvious solution. Minnesota stands well among her sister States in her equipment and organization for the sanatorium care of her consumptives. But her programme is not complete because she has no working colony. We must not be content to look back on our achievements, but must constantly be on guard lest our boast that we are among the leaders in this work be turned into our shame that we are allowing others to pass us, while we hug to our bosoms the comforting thought that we have in the past been leaders.

For post-sanatorium supervision, Wisconsin has for years been building up a working colony for men at Tomahawk Lake, and quite recently it was my privilege to dine with a gentleman who is building a working colony, at his own expense, for the sanatorium graduates of Michigan.

Sheltered employment is offered by the Altro Manufacturing Company in New York City, and by many sanatoria throughout the country which employ ex-patients in the institutions, or on the grounds or farm.

Papworth Colony, Cambridgeshire, England, is a very excellent example of an industrial colony for such cases. The ex-patients live in large boarding houses or in separate family homes. In the latter the family of the sufferer joins him and the family life is reëstablished. Work can be had by the able-bodied individuals, who thus become an integral part of the colony. In the workshop the leather-worker, the wood-worker, the book-binder, the printer, the jeweler, and so forth, are all given an opportunity to carry on the work which they have been trained to do, and at which they are most able to earn good wages. Some work on the farm and others are engaged in raising chickens. The amount of work is prescribed according to the physical condition of each individual and is very gradually increased as the months go by.

Ex-patients are encouraged to live in the colony until they are able to do a full day's work and are fully equipped to reënter the industrial world without any handicap or the necessity of asking for preferential treatment.

The colony is headed by Dr. Varrier-Jones and policed by his trained workers. The nurses are in every department, and constant temperature records

are kept. Anyone having any suspicious symptoms is at once sent to the sanatorium for further observation and treatment.

Such an institution is necessary to round out and complete Minnesota's armament in this fight against tuberculosis, and the State Board of Control, now the mentor of Minnesota's tuberculosis work, should put it down as the next necessary step in their programme.

ARTIFICIAL PNEUMOTHORAX

With a Report of 150 Cases[1, 2]

P. M. MATTILL AND M. H. WALL

The data that form the basis of this report have been derived from a study of 150 of the cases entering Glen Lake Sanatorium (Minnesota) prior to January 1, 1925. The work was done by members of the Glen Lake staff, extending over a period of about eight years. A standardized technique has been developed and is used uniformly in all cases.

For the sake of convenience, the cases will be considered in three groups:

1. Patients with no free pleural space
2. The patients in whom treatment was discontinued
3. Patients now under treatment

All those doing pneumothorax find a considerable number of cases in which collapse of the lung is impossible because of adhesions. It is generally agreed that adhesions head the list of all the causes of failure of artificial pneumothorax. In general, the prognosis is not so good in these cases because the extensive adhesions, in most of them, usually mean extensive disease of long duration. Many would go on to a fatal termination unless the diseased lung were collapsed. Coöperation with the surgeon in obtaining a collapse has given a chance for recovery to many a patient that otherwise would have died. In our group of 37 cases, in which collapse was impossible by pneumothorax, it has been produced by thoracoplasty in 19, or over 50 per cent.

Of those subjected to thoracoplasty, four have died, eleven are still receiving treatment and four are working. Of those not operated upon, 18 cases, seven are dead, ten are in the Sanatorium with operation advised in three cases, and one is working. (Table 1.)

Fifty-two cases in the second group were given pneumothorax for varying periods of time (table 2). Twenty-seven of these are dead and

[1] From the Glen Lake Sanatorium, Oak Terrace, Minnesota.

[2] Read before the Clinical Section at the twenty-first annual meeting of the National Tuberculosis Association, Minneapolis, Minnesota, June 17–20, 1925.

25 are living. Of those who have died a satisfactory collapse was obtained in 22, or in 81 per cent of the cases. But they were not benefited except temporarily, because of tuberculous or nontuberculous complications, or because of extension of the pulmonary process. The average time of treatment was ten months. There were tuberculous complications in 13 and nontuberculous in four, making a total of 66 per cent having complications. There was fluid in 17 cases, 5 of which terminated in empyema. Two with good collapse and good prognosis left the institution against advice. Of the five having an unsatisfactory collapse, one had a thoracoplasty, two others had serious complications, and two left against advice who might have been benefited by further sanatorium treatment.

TABLE 1

NUMBER OF CASES	DEAD	LIVING	THORACOPLASTY DONE	TREATMENT		WORKING
				Sanatorium	Home	
19	4	15	19	11		4
			THORACOPLASTY ADVISED			
18	7	11	3	9	1	1
37	11	26		20	1	5

TABLE 2

NUMBER OF CASES	DEAD	LIVING	COLLAPSE		DURATION OF TREATMENT IN MONTHS	COMPLICATIONS			
			Satisfactory	Partially satisfactory		Nontuberculous	Tuberculous	Fluid	
								Serous	Empyema
27	27		22	5	10	4	13	17	5
25		25	20	5	16		7	16	2
52	27	25	42	10		4	20	33	7

Of the 25 cases living, in which pneumothorax was discontinued, collapse was satisfactory in twenty, or in 80 per cent of the cases. The average time of treatment was sixteen months. It was discontinued for the following reasons: Extension in the contralateral lung, 7 cases; unsatisfactory collapse, 4 cases; left the institution against advice, 5 cases; discharged improved, 2 cases; unfavorable reaction, 3 cases; empyema, 2 cases; disease arrested, 2 cases. Sixteen are still receiving treatment in or out of the Sanatorium.

It should be noted that in spite of a satisfactory collapse in over 80 per cent of this group, with a relatively prolonged period of treatment, the benefits derived were more or less temporary. Of those still living, 64 per cent still require medical care. Extension of the disease and complications, tuberculous and nontuberculous, are the important factors. This should not lead to the conclusion that complications, especially those of a tuberculous nature, are contraindications to pneumothorax; rather, they indicate that the disease is so widespread that collapsing the lung would lead to no permanent benefit. In general, in the presence of complications the patient's prognosis should be better with the diseased lung collapsed.

TABLE 3

NUMBER OF CASES	COLLAPSE		TIME OF TREATMENT IN MONTHS	COMPLICATIONS			EXTENSION	BILATERAL PNEUMOTHORAX	PRESSURE		
	Satisfactory	Partially satisfactory		Tuberculous	Fluid				Positive	Mean zero	Negative
					Serous	Empyema					
14	14		29	1	8	0	1		8	3	3
47	39	8	15	14	24	2	13	6	18		29
61	53	8		15	32	2	14	6	26	3	32

There are 61 cases in group 3 now under treatment (table 3). Forty-seven are in the Sanatorium, and 14 are receiving treatment on the outside. Of those in the Sanatorium 39 cases, or 83 per cent, have a satisfactory collapse and 8 have a partially satisfactory collapse. The average time of treatment has been fifteen months. There are tuberculous complications in 14, fluid in 24 with a complicating empyema in two cases. There has been an extension of the disease in a total of 13 cases; six of these have been given bilateral pneumothorax with definite benefit in each case. All of the cases receiving treatment on the outside have a satisfactory collapse, and were discharged improved. Of those discharged, one had an extension in the good lung before leaving the institution and 8 had fluid. The average time of treatment was twenty-nine months.

In the entire group it is of interest to note that a definite positive pressure was necessary in 26 cases, a mean zero pressure in 3, and a negative pressure in 32. These positive pressures are necessary for the most part to maintain a collapse, when there is a tendency to obliteration of the pleural space following the formation of fluid. Positive

pressures are used in some cases to compress a lung held out by bands of adhesions or for the purpose of collapsing a cavity. Whenever possible, collapse of the lung should be maintained with negative intrapleural pressures. This should be done with relatively small amounts of air given as frequently as is found necessary.

The majority of those now receiving treatment are being definitely benefited. A large number should go on to recovery, but treatment has not been continued for a sufficient time to know how many will do so. Thoracoplasty should be done in some of those in whom pneumothorax is only partially satisfactory. In an additional small group of cases, in which it is evident that collapse must be permanent, it would seem to be the part of wisdom to produce that collapse by operative

TABLE 4

INDICATIONS FOR COLLAPSING LUNG	NUMBER	PER CENT
Extensive involvement (with or without cavity)	61	41
Extension of disease	26	17
No improvement	24	16
Treatment began before entrance	17	11
Hemorrhage	15	10
Air to replace fluid	7	5
Total	150	

procedure as soon as the condition of the patient would warrant. Pneumothorax is not without some risk from fluid, empyema, and other less frequent complications, and there is always the danger of the patient discontinuing treatment. A permanent collapse would obviate these dangers.

The indications for the institution of pneumothorax in this series are given in table 4. It will be observed that extensive involvement with or without cavity leads the list, whereas hemorrhage is one of the less frequent indications. It is not used in early cases except for the control of hemorrhages, and these cases are comparatively rare. A criticism may be made that in many cases treatment was delayed too long, but, since in general such a large number recover without the use of pneumothorax, the usual conservative treatment should be tried first. The extended period of time required for pneumothorax treatment must be taken into account in starting treatment in relatively early cases.

The condition of the contralateral lung requires careful study. Ideally, this should be entirely clear both by physical signs and X-ray. For the entire series (table 5) the contralateral lung has been clear in 27 per cent of the cases; whereas, for the group under treatment at the present time, it is entirely clear in 43.5 per cent, indicating a more careful selection. In determining the condition of the contralateral lung, the X-ray is absolutely necessary. Many cases which have no definite physical signs show the presence of infiltration by X-ray. Our experience has been that, unless there is evidence of definite fibrosis, the danger of the lesion becoming activated is very real. The opinion of our roentgenologist is obtained in all cases. There are a few exceptional cases in which physical signs clear up with the collapse of the more extensively diseased lung. In general, it may be said that signs of activity in the good lung, as determined by physical examination and recent parenchymal lesion without phyical signs as seen by X-ray, are definite contraindications for collapsing the more extensively diseased lung.

TABLE 5

CONTRALATERAL LUNG (X-RAY)	NUMBER	PER CENT
Entirely clear	41	27+
Fibroid tuberculosis	59	39+
Fibroid parenchymal	42	28
Calcified	5	3+
Peribronchial	3	2+
Total	150	

Fluid is the most frequent complication, being present in greater or less amount in slightly more than 50 per cent of all the cases. Empyema followed fluid in about 8 per cent of all the cases. Formation of fluid is in itself not of serious consequences. When the amount is small it would frequently escape notice unless seen by the fluoroscope or X-ray. In a certain number of cases repeated aspirations must be done, and the fluid persists for months. The danger is that with the absorption of the fluid there will be an obliteration of the pleural space. When the fluid becomes purulent the prognosis becomes less favorable. Up to this time various methods of treatment have been resorted to in our cases of empyema without great success.

Other less frequent complications are spontaneous pneumothorax, three cases; generalized subcutaneous emphysema, one; air embolism,

one; and puncture of parenchyma with the spitting of blood in numerous instances.

A summary of all the cases (table 6) shows the admission diagnosis to be far advanced, 66; moderately advanced, 82; and incipient, 2. Thirty-eight have died and 112 are living. Of those living, 80 are receiving treatment in the Sanatorium and 14 are at home. Of one there is no record, while 17 are working.

The above summary gives an inadequate idea of the value of pneumothorax if the value is to be measured in terms of end-results. Relatively few cases have become arrested, but many have had and are

TABLE 6

	FAR ADVANCED	MODERATELY ADVANCED	INCIPIENT	TOTAL	DEAD	LIVING	UNDER TREATMENT		NO RECORD	WORKING
							Sanatorium	Home		
Group 1										
Men	9	7		16	11	26	20	1		5
Women	11	10		21						
Total	20	17		37						
Group 2										
Men	9	11	2	22	27	25	13	3	1	8
Women	14	16		30						
Total	23	27	2	52	27					
Group 3										
Men	6	12		18		61	47	10		4
Women	17	26		43						
Total	23	38		61						
Grand total	66	82	2	150	38	112	80	14	1	17

having their life prolonged. They are enjoying a degree of health and well-being which would not be possible otherwise; and, as time goes on, an increasing number of those now under treatment should go on to recovery. A better selection of cases, with the increasing knowledge concerning the application of the treatment, will continue to make artificial pneumothorax one of the outstanding methods in the treatment of pulmonary tuberculosis.

SUMMARY

1. Pneumothorax should be supplemented by thoracoplasty in those cases having no free pleural space.

2. Thoracoplasty should be considered in those cases in which pneumothorax is only partially satisfactory and in those cases in which a permanent collapse is indicated.

3. Pneumothorax may be of limited value because of tuberculous or nontuberculous complications.

4. Fluid is the most frequent complication, having occurred in about 50 per cent of our cases.

5. Pneumothorax is not indicated in the early cases. In the more advanced cases it should not be done before giving the usual sanatorium treatment a trial, except in selected cases.

6. The contralateral lung, ideally, should be entirely clear. Healed or well-fibrosed lesions may be present, but evidence of activity, as determined by physical examination or recent infiltration as seen by X-ray, are definite contraindications in most cases.

7. Negative pressures should be used whenever possible. Positive pressures are warranted to maintain collapse when there is a tendency to obliteration of the pleural space.

8. The true results of pneumothorax are not shown by the few cases in which arrest of the disease has been obtained.

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THE PATHWAYS FOR NERVOUS REFLEXES FROM THE PARENCHYMA OF THE LUNG[1]

A. T. RASMUSSEN[2]

With the more accurate knowledge of the nerve supply to the lung which is now available, especially through the work of Larsell (1921–1924), it is possible to arrive at a fairly definite conclusion regarding the probable neurological paths underlying the reflexes and referred sensations associated with pathological involvement of the lung.

The afferent nerves with which we are here especially concerned are found in three principal places, (1) in the walls of the bronchial tree, (2) along the pulmonary artery, and (3) in the visceral pleura. All these nerves enter the lungs in the region of the hilum.

The nerves in the walls of the bronchi form two plexuses, one superficial to the cartilage and the other beneath the cartilage. In the smaller bronchioles, where no cartilage is found, these two plexuses are fused into a single plexus which continues as far as the alveolar duct. Nerves from these plexuses supply the epithelium of the mucous membrane, the plain muscle and glands of the bronchi. The sensory nerves to the epithelium of the mucous membrane terminate, in general, as free nerve endings, but in the region of the atria, where the epithelium is squamous, there are flattened sensory corpuscles just under the epithelium. Special masses of nerves are evident at the crotch between the two branches of a dividing bronchus, where they might be stimulated by a decrease in the angle between the bronchi, as occurs in expiration. At these points are also aggregations of lymphoid tissue which enlarge in tuberculosis and other infections, and thus may irritate the afferent nerves in this neighborhood.

Afferent nerve fibres also enter the muscle of the bronchi, where they terminate in special endings called *smooth muscle spindles* by Larsell.

Sensory corpuscles have also been demonstrated in the larger pulmonary arteries (Larsell, 1921–1923; Dogiel, 1898).

[1] Presented by invitation before the Medical Staff of the Lymanhurst School for Tuberculous Children, January 23, 1925.

[2] Professor of Neurology, Department of Anatomy, University of Minnesota, Minneapolis.

Nearly all these afferent nerves (except those to the pleura) degenerate in a particular lung when the corresponding vagus is cut (Larsell and Mason, 1921), indicating that they come almost entirely from the vagus of the same side. A few come either from the other vagus or from the thoracic spinal nerves.

The vagus, as is well known, carries important afferent impulses from the lung to the respiratory centre in the medulla oblongata, although physiological experiments (Pike and Coombs, 1922, and others) indicate that the thoracic spinal nerves also carry afferent impulses that influence respiration.

The nerves which go to the visceral pleura are composed of large medullated fibres which terminate largely in the interlobular region in various forms of sensory corpuscles. Great areas of the visceral pleura in contact with the chest-wall are, however, practically devoid of nerves. Experiments on nerve degeneration by Larsell show conclusively that these nerves come from the upper thoracic spinal nerves.

In brief, the afferent fibres of the lung are derived from two separate sources: (1) the vagus, which supplies the interior lung parenchyma, and (2) the upper thoracic spinal nerves, which supply restricted regions of the pulmonary pleura (see accompanying figure). Reflexes from the lungs must, therefore, be carried back to the central nervous system by these two routes, though chiefly through the vagus, since practically all the afferent fibres of the interior of the lung are supplied by this nerve. The muscular spasticity and referred sensations resulting from pulmonary tuberculosis and other infections of the lung with no involvement of the pleura must, therefore, be due to reflexes in which the afferent limb of the reflex arc is largely or entirely in the vagus. And, on the other hand, when the pleura is primarily involved, the afferent limb of the reflex arc must be chiefly in the thoracic spinal nerves. Lesions in the hilum of the lung, where nerve fibres from both sources enter, naturally may easily irritate fibres of both the vagus and thoracic spinal nerves.

These statements mean very little unless the pathways to those regions of the central nervous system that act as reflex centres are followed up. The sensory fibres of the vagus enter the upper portion of the medulla oblongata, where some terminate in the immediate neighborhood while others run downward toward the spinal cord as part of the solitary fasciculus to end in various centres, especially the respiratory centre. A few fibres may even go down into the spinal cord as far as

the fourth cervical segment (Cajal). Freeman (1925) has also described a bundle of fine fibres as far as the third cervical segment. All along the solitary fasciculus is a column of nerve-cells, known as the nucleus of the solitary fasciculus, which relay the impulses to various secondary centres and to motor nuclei of cranial and spinal nerves. In one or both of these ways the midcervical region of the spinal cord is evidently very closely associated with afferent nerve fibres from the lung, since no afferent nerve fibres have been demonstrated to enter this region of the spinal cord by way of cervical nerves. An abnormal number of impulses transmitted to the gray matter of the midcervical segments of the cord, as a result of inflammation or other irritating conditions in the lung, may stimulate the nucleus of the spinal portion of the accessory or eleventh cranial nerve, which arises from a long column of cells in the upper half of the cervical cord. This is the most probable explanation for the spasticity of the sternocleidomastoid and trapezius muscles in tuberculosis of the lung, since the lesion starts in the interior of the lung rather than in the pleura. In a similar manner the motor cells of the phrenic nerve are activated, causing increased tone in the diaphragm, which in turn decreases the amount of diaphragmatic excursion so common in pulmonary tuberculosis.

This constant excessive stimulation of the midcervical region evidently accounts also for the referred pain located in the cutaneous areas supplied by the midcervical nerves.

The other route from the lung, which is especially connected with the pulmonary pleura, involves fibres which pass from the lungs to the lower cervical and upper thoracic sympathetic trunks, and out through the upper white *rami communicantes* to the upper thoracic nerves, and thence into the upper thoracic regions of the spinal cord over the corresponding dorsal roots. After entering the spinal cord some of these fibres ascend to the midcervical region and higher levels, and others terminate about the association cells in the gray matter near the level of the entrance into the spinal cord. These association cells in turn relay impulses especially to the midcervical region, but also to higher and lower levels (see accompanying figure).

Since the sensory disturbances resulting from pulmonary diseases are so often associated with the cutaneous areas supplied by the middle cervical nerves and with the muscle reflexes largely confined to muscles supplied by motor cells in the upper half of the cervical cord, the tendency has been to assume that the afferent impulses from the irritated

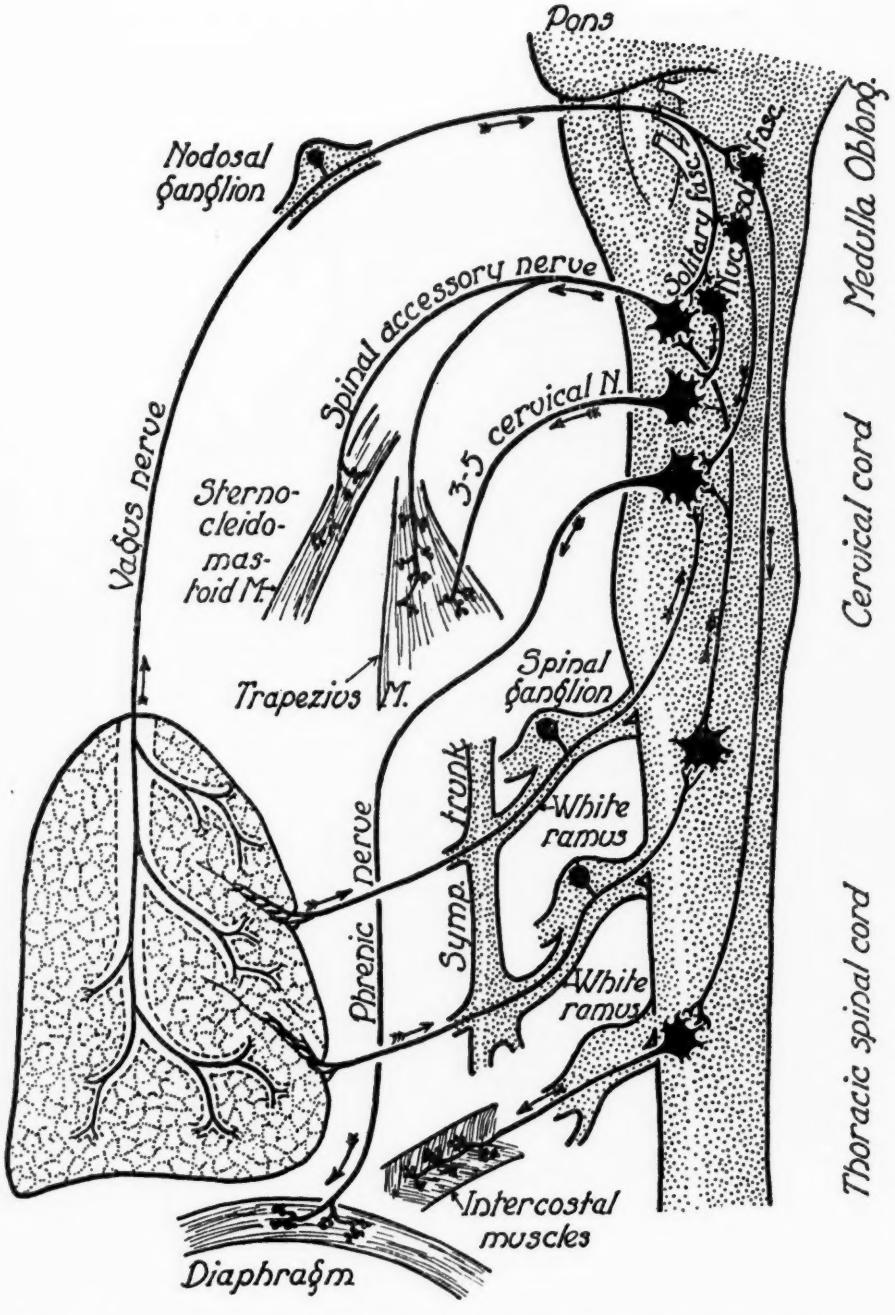

DIAGRAM SHOWING THE COURSE OF AFFERENT IMPULSES FROM THE LUNG PARENCHYMA VIA THE VAGUS AND ALSO THE COURSE OF AFFERENT IMPULSES FROM THE PULMONARY PLEURA VIA THE SYMPATHETIC SYSTEM AND UPPER THORACIC NERVES TO THE CERVICAL REGIONS OF THE SPINAL CORD AND MEDULLA OBLONGATA

lung must enter the spinal cord over the cervical nerves. While many reflexes and referred sensations are strictly segmental, this is not invariably the case, as an examination of many pelvic diseases, especially, will show. Many normal reflexes are very far from being segmental. Note the dilator eye reflex which passes down from the midbrain, where the afferent impulses enter, to leave the spinal cord over the upper thoracic nerves.

The important facts in connection with the probable pathways for the referred sensations and motor reflexes from the lungs are the anatomical and physiological findings that the afferent fibres from the pulmonary pleura are derived almost entirely, if not entirely, from the thoracic nerves, and that the afferent fibres to the bronchial structures are almost exclusively from the corresponding vagus. Until more direct afferent courses are actually demonstrated, the scheme here outlined (see figure) must be looked upon as the most probable pathways for the reflexes from the lungs, the vagus route being especially important when the pleura is not involved, as in early stages of pulmonary tuberculosis.

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AN INVESTIGATION OF THE ACID-FASTNESS OF TUBERCLE BACILLI

Second Report[1, 2]

B. SUYENAGA

In a previous report (AMERICAN REVIEW OF TUBERCULOSIS, *1925, xii, 260*) I concluded that the establishment of a non-acid-fast culture from the K1 strain of tubercle bacillus, by rapid transfer and rapid growth of the border or younger portion of its colonies, was impossible. The metabolic conditions of the culture were changed by using different reactions, which, however, had no marked effect on the acid-fastness. The non-nutrient medium and the amoeba medium used by Wherry practically robbed the culture of its acid-fastness, but the bacilli restored it again after having been kept for a long time on these media. This K1 strain has been generally thought to be a true human strain of tubercle bacilli originally isolated in the laboratory of Robert Koch, and its non-acid-fast element has attracted our close attention.

It is probable that young tubercle bacilli are less acid-fast than old microörganisms, or are even non-acid-fast, for in my previous work a reduction of acid-fastness of K1 was effected by rapid transfers of its young growth. It was also found that a non-acid-fast element was demonstrable in the culture of virulent strains. However, the possibility of symbiosis of a non-acid-fast bacillus with the acid-fast germ, or a mutation strain appearing in this culture, suggested itself. The smears from K1 culture often show acid-fast and non-acid-fast bacilli in separated groups. Another interpretation of the non-acid-fast bacilli of K1 culture is that they are degenerated forms. Unna once asserted that the non-acid-fast tubercle bacilli are the dead ones. This is highly improbable, especially in the case of K1. The non-acid-fast bacilli in this culture are counterstained well and uniformly by methylene

[1] From the Otho S. A. Sprague Memorial Institute and Pathological Laboratory of the University of Chicago.

[2] A summary of this work was read at the annual meeting of the National Tuberculosis Association at St. Louis, Missouri, in 1920, and was published in THE AMERICAN REVIEW OF TUBERCULOSIS, 1920, iv, 526.

blue, and the outlines of the bacillary bodies are clean-cut. Their sizes are regular and normal, without showing any sign of degeneration.

The idea of mutation and symbiosis in K1 cultures became more and more improbable as this work progressed. For instance, we learned, when a number of other supposedly acid-fast, saprophytic bacilli, resembling the K1 culture in many respects, were carefully stained and examined, that a great many non-acid-fast bacilli were found in smears from their cultures. It seems improbable that this phenomenon in all of the various strains examined could be due to mutation or symbiosis. However, this work was undertaken to eliminate these possibilities as an explanation for the non-acid-fast finding in K1 cultures. For this purpose the experiments described below were conducted with the use of chemicals which would act differently on the acid-fast and non-acid-fast forms, in order to distinguish and separate the one type from the other if possible. The work further sought to see whether the non-acid-fastness of younger tubercle bacilli could be more definitely established.

The experiment was extended in connection with chemotherapeutic subjects, when gentian violet was tried as a means of separating the two elements in K1 cultures. The results of this part of the work are described under the heading of *experiment 2* in this report.

EXPERIMENT 1

1. Efforts to Separate the Acid-Fast and Non-Acid-Fast Bacilli in K1 Cultures: One of the methods used for this purpose was the application of the principle of isolation of tubercle bacilli from sputum. It was possible that sodium hydroxide would destroy all non-acid-fast bacilli, and leave the acid-fast microörganisms in K1 cultures. Thus it had to be seen whether the latter would remain acid-fast after such treatment.

The K1 culture was exposed, at first, to a 1.5 per cent solution of sodium hydroxide. This was done by adding 3 per cent sodium-hydroxide solution to an equal amount of a filtered, thick suspension of a two-day-old K1 culture in physiological salt solution. The duration of these exposures was from ten minutes to five hours. The mixtures, after each desired time of exposure, were neutralized to litmus paper with 1/3 normal hydrochloric acid and then centrifugated for 20 minutes and decanted. One loopful fo the sediment was inoculated on glycerol-agar slants, two loopfuls on Petroff medium containing 1:10,000 of

gentian violet, and six loopfuls on the same medium with 1:1,000 of gentian violet. For all ten-minute sets only one loopful was inoculated. Only one of three glycerol-agar slants, transferred after 30 minutes' exposure, was found to have grown the colonies of K1 after four weeks of incubation. This growth contained both the acid-fast and non-acid-fast elements. The work was repeated, 0.5 per cent solution of sodium hydroxide having been used this time. The bacillary suspension of K1 was mixed with the same amount of 1 per cent sodium-hydroxide solution. Three loopfuls of the treated sediment were inoculated. All the growths obtained after incubation were tested for acid-fastness, and they proved to contain both acid-fast and non-acid-

TABLE 1

Effect of exposure to sodium hydroxide on growth of tubercle bacilli

TIME OF EXPOSURE	K1 EXPOSED TO 1.5 PER CENT NaOH			K1 EXPOSED TO 0.5 PER CENT NaOH		A VIRULENT STRAIN EXPOSED TO 0.5 PER CENT NaOH	
	Glycerol agar	Petroff's medium (G.V. 1:10,000)	Petroff's medium (G.V. 1:1,000)	Glycerol agar	Petroff's medium (G.V. 1:10,000)	Glycerol agar	Petroff's medium (G.V. 1:10,000)
10 minutes	− − −	− − −	− − −	+ + +	+ + +	− − −	+ + +
30 minutes	− + −	− − −	− − −	+ − −	+ + −	− − −	+ + +
1 hour	− − −	− − −	− − −	+ + −	+ + −	− − −	+ + +
2 hours	− − −	− − −	− − −	− − −	− − −	− − −	+ + +
3 hours	− − −	− − −	− − −	− − −	− − −	− − −	+ + +
5 hours	− − −	− − −	− − −	− − −	− − −	− − −	+ + +

Note: The final record was taken over two months after the incubation. G.V. = gentian violet.

fast bacilli. The tabulated results were recorded 47 days after the incubation (table 1). Coincidently, a filtered, thick suspension of a culture of a virulent strain was treated in the same manner, and the results of incubation for 29 days, which remained the same for another month, are also given in table 1. The virulent culture stood the alkaline reagent very much better than the K1 culture, which was completely destroyed after exposure for two hours to a 0.5 per cent sodium-hydroxide solution. The virulent culture grows only on Petroff medium when in suspension. This is also true with the untreated suspension. Three tubes each of glycerol-agar and Petroff medium were inoculated with one drop of a suspension of the virulent culture. On the 15th day of incubation, all the Petroff media had grown the tubercle bacilli, while glycerol agar had no growth even until the 61st day.

The second method in the effort to separate two types of bacilli in K1 culture was by the use of gentian violet. This was tried because of its special selective action on gram-positive microörganisms. This action of gentian violet and its allied dyes was emphasized by Churchman, and was called a *bacteriostatic* action. Gram-positive microörganisms fail to grow on media dyed even very weakly with gentian violet. According to Tamura (*Zeitschrift für physiologische Chemie, 1913, lxxxvii, 85*), the acid-fastness of tubercle bacilli is due to a higher alcohol termed *mykol*, and this substance is also gram-positive. So it is reasonable to suppose that a certain strength of gentian violet might act specifically on acid-fast or gram-positive bacilli, or both, in the case of K1 culture, and that the gram-negative forms would survive them. Or, acid-fasts, owing to their high resistance, would be left intact alone. From the inhibitory and bactericidal experiments to be described later in detail, it became known that a 1:10,000 dilution of gentian violet did not inhibit seriously the growth of K1 on plain nutrient agar (table 2). Therefore, agar slants containing this strength of gentian violet were inoculated with a thin and fine suspension of K1 culture. Transfers were made every three or four days until the fifth generation. Then two more transfers were made on 1:5,000 gentian-violet agar. The culture then was examined, and was found still to contain both acid-fast and non-acid-fast bacilli. The same result was obtained when various strengths of gentian violet and methylene blue were used. These experiments are reported in detail further on in this paper. Thus a mutation strain or symbiosis in K1 culture became decidedly improbable. The work was then directed toward testing of the hypothesis of non-acid-fastness of young tubercle bacilli. The first step to be taken in this direction was to search for non-acid-fasts in virulent cultures.

2. Non-Acid-Fast Bacilli in Virulent Cultures: It was shown in my previous report on this subject that there were a number of bacilli stainable by a watery solution of methylene blue in the smears from virulent cultures of tubercle bacilli. In the present report two more or less virulent strains of tubercle bacilli at different stages of growth were studied for their non-acid-fast content by the Ziehl-Neelsen stain. The smears for staining were taken from the border growth of the colonies. The cultures used and their ages when examined were as follows:

A. A virulent human strain, newly isolated and cultured on glycerol agar, 3, 5, 8, 14, 17, 21, 26, and 33 days old.

B. A weakly virulent human strain isolated many years ago, when 5 and 9 days old on glycerol agar.

C. The same strain as *B*, when 5 and 7 days old on glycerol broth.

Although Klein (see study I) found the non-acid-fast element of tubercle bacilli cultivated on horse serum only when they were younger than two weeks, I found a number of them even after they were more than four weeks old. The weakly virulent strain contained the non-acid-fast element considerably more than the virulent strain. However, the number of non-acid-fast bacilli was slight as compared with the acid-fast and, further, the perfectly non-acid-fast bacilli were fewer. The presence of these very few non-acid-fasts in the virulent culture apparently contradicts the hypothesis that the youngest tubercle bacilli are always non-acid-fasts. Yet the two seem to be compatible only if the non-acid-fast period of the tubercle bacillus is short. The slow development of tubercle bacilli is well known and the following gross calculation of the speed of their multiplication may be helpful.

Five tubes each of a glycerol agar culture of two virulent strains which were three weeks old were used for this purpose. First, the estimated amount of material for inoculation was weighed for each tube, and then the rest of the colonies in the tube were added to it and the whole was weighed again. The average amount of inoculated material for all ten culture tubes was 9.5 mgm. The average amount of colonies developed in three weeks in a whole tube was 164 mgm. for one strain (strain 1305) and 122.2 mgm. for the other strain (strain 461). Therefore, the former multiplied 17.3 times and the latter 12.9 times the inoculated amount, or an average of 15.1 times in three weeks. The five culture tubes of each strain used for the weighing were of the most luxuriant growth among ten culture tubes of the same strain inoculated in the beginning. The multiplication of fifteen times in three weeks might be interpreted grossly as one cell division in five days. We do not know the process of cell division of tubercle bacilli in both chemical and mechanical detail. But it is conceivable that the physical structure and chemical composition of a tubercle bacillus is different in the younger stage of development of its individual life from those in the more matured stage, and that the cell division in the tubercle bacillus takes a much longer time than in the case of other bacilli.

The very short period of non-acid-fastness of tubercle bacilli is compatible with the reported success in obtaining some slight reduction

in the acid-fastness of the rapidly growing K1 culture by rapid transfers of its border growth. This very short period of non-acid-fastness is conditional on the hypothesis that the young acid-fast bacillus is non-acid-fast.

3. A Comparison of the Degree of Acid-Fastness of the Central and Marginal Growths of K1 Colonies: If the acid-fast microörganisms, including the tubercle bacillus, are non-acid-fast in their youngest stage, the border growth which represents the spreading, younger part of the culture should prove to be less acid-fast than the central part of the colonies. Therefore, a comparative examination was made of these two parts for acid-fastness, using the K1 cultures on our laboratory glycerol agar, on glycerol agar of various reactions, amoeba medium and non-nutrient agar. The 190th generation of the rapid-transfer culture studied in the first report was also tested. There were 49 examinations of one to seven-day-old cultures. The staining was done with the Ziehl-Neelsen method as in my first report. The time for decolorization in the 14 per cent nitric-acid solution was from 30 seconds to 10 minutes. Three minutes in 70 per cent alcohol was constant all through these tests. The following results were obtained.

	Marginal growth	*Central growth*
Decidedly less acid-fast than the other part of growth	16	7
Only slightly less acid-fast than the other part of growth	9	4
The number of instances in which no difference of acid-fast degree was noticeable	13	

The results of the above experiments favor the hypothesis that the tubercle bacillus is non-acid-fast in its youngest stage.

EXPERIMENT 2. INHIBITORY AND BACTERICIDAL ACTION OF GENTIAN VIOLET AND METHYLENE BLUE ON ACID-FAST AND OTHER MICROÖRGANISMS

When gentian violet was used in an effort to separate the two elements of K1 cultures, it was necessary to find out the maximum strength of gentian violet which would not inhibit the growth of the cultures, that is, the initial tolerance of K1 culture to gentian violet. At this stage of the work the experiments with gentian violet and also with methylene blue were extended to the chemotherapeutic field, involving a number of other acid-fast and non-acid-fast microörganisms.

The useful tinctorial character and a certain selective antiseptic action of gentian violet have long been known in bacteriology. How-

ever, Churchman gave special prominence to this dye and its allied dyes when he reported (*Journal of Experimental Medicine, 1912, xvi, 221; 1913, xvii, 373; 1913, xviii, 579*) his extensive studies on their selective *bacteriostatic* action on gram-positive microörganisms. Churchman also found that methylene blue had the same selective bacteriostatic action, but it was not always sharply manifested. DeWitt, in her chemotherapeutic work with various dyes, took a special interest in methylene blue. She studied a number of preparations of methylene blue, both *in vitro* and *in vivo*, and found a considerable inhibitory and bactericidal action of methylene blue for tubercle bacilli (Wells, DeWitt and Long, *The Chemistry of Tuberculosis*, pp. 362–366). Churchman's work has been followed by many investigators who have studied the above-mentioned and many other dyes for various purposes. Among them, the work and the review of the literature regarding gentian violet and other dyes by Krumwiede and Pratt (*Journal of Experimental Medicine 1914, xix, 20*), Hall and Taber (*Journal of Infectious Diseases, 1914, xv, 566*), and Crossley (*Journal of the American Chemical Society, 1919, xli, 12*) are some of the representative reports. Hall and Ellefson reported the advantageous use of gentian violet in their *Elimination of Spurious Presumptive Tests for B. Coli in Water* (*Journal of Bacteriology, 1918, iii, 329*), and in *Milk with Reference to Sporulating Anaerobes* (*Ibid. 1918, iii, 355*). Churchman (*Journal of the American Medical Association, 1921, lxxvii, 24*) and others have also been developing the use of gentian violet for surgical conditions. Smith and Casparis (*Journal of the American Medical Association, 1923, lxxxi, 2184*) were successful in treating a case of staphylococcus septicemia with gentian violet. Their preliminary work with this drug made their report more convincing.

Churchman divided a vast number of microörganisms into two great groups of gentian-positive and gentian-negative bacteria. Generally speaking, gentian-positive forms are gram-positive bacteria; they fail to grow on agar dyed with gentian violet. Gentian-negative microorganisms are gram-negative bacteria and grow on gentian-violet agar. There are four strains of tubercle bacilli and three other acid-fast saprophytes in the list of the numerous microörganisms studied by Churchman. The growth of all these was irregular and often delayed on gentian-violet agar. The technique of his method will be described in the following experimental part of this report.

In my experiments, acid-fast bacilli, most of which were saprophytes, were grown on gentian-violet media and methylene-blue media with or

without glycerol. The reactions of these microörganisms to various strengths of the two dyes were studied with the hope that the acid-fasts would show something interesting as compared with other gram-positive microörganisms, or that there might be manifested some particular reaction to the dyes in some of these microörganisms.

1. Inhibitory Experiment

Churchman's divided-plate method was followed in this experiment. Petri dishes, each of which was divided into two compartments by a strip of German silver, were sterilized. The metal strip was cut a little longer than the diameter of the dish, and its breadth was about 0.5 cm. Therefore, after this metal strip was fixed in the dish by its elasticity, the height of the septum which divided the two compartments was 0.5 cm. The melted and sterilized agar was aseptically poured into one compartment to the height of the metal septum; after it was cooled and solidified, the other compartment was similarly filled with the melted, dyed agar. Thus the dyed and plain agar made a smooth surface across the metal septum.

One per cent watery solution each of gentian violet (Grübler) and methylene blue (Grübler, rectif. Ehrlich) was used as stock solutions for dying agar. These stock solutions were heated in an Arnold sterilizer at 100°C. for over one hour. This step helped to dissolve the dyes satisfactorily. Then 100 or 200 cc. each of various strengths of gentian violet and methylene blue in agar were prepared from them. The divided plates were prepared in turn with the dyed agar and plain agar. In melting the agar overheating was carefully avoided.

Bouillon cultures of the microörganisms to be tested were made after rejuvenating the stock cultures. A number of such cultures had not developed uniformly. Therefore, all broth cultures studied at any one time were made of equal turbidity by diluting with the plain or glycerol broth according to the experiment. This was done after the bouillon cultures were filtered through sterilized cotton or several sheets of gauze in a funnel. Thus the better developed culture tubes were diluted to the same turbidity as that of the least developed tubes. When K1 was tested together with other non-acid-fast microörganisms, K1 in broth showed the poorest development after about 24 hours in the incubator. In this case the broth cultures of other microörganisms were diluted to the turbidity of K1 cultures. The film formed on broth cultures of

acid-fast microörganisms was stirred up, so as to make a uniform suspension. For the slow-growing group among the acid-fasts[3] (avian tubercle bacilli, frog bacilli, leprosy bacilli Duval) experimented on along with K1, three-day-old cultures in glycerol media were used.

A large smooth platinum loop was used for transferring such filtered and standardized bacterial suspensions, a loopful of each suspension being lightly drawn over the plate of solidified and finished agar, perpendicular to the septal metal, making the streaks on the plain and dyed part a straight, even clean line. The streak was made on the plain agar side and then on the dyed side. One dish had three such lines, of different microörganisms,—one in the middle, and one on each side. For one set of experiments, from four to seven kinds of microörganisms were used, and the combination of three species in one dish was so made that the suspension streak of a microörganism would appear on three or four plates along with other microörganisms. This made comparative observations of growth of the microörganisms on the divided plates feasible.

Four sets of inhibitory experiments were conducted. First, three were done with the use of plain and dyed nutrient agar, and the fourth with glycerol and dyed glycerol agar. After inoculation, the dishes were incubated, and observations were made at definite intervals. The tables made from these records show the net results.

As seen in table 2, the acid-fast K1, timothy, grass, mist and smegma bacilli are inhibited by strengths of gentian violet from 1:10,000 up in plain agar, while *B. coli* and *B. prodigiosus* are affected only by 1:1,000 dilutions. Both *B. subtilis* and *Staph. aureus* are inhibited by even a 1:100,000 dilution of gentian violet. Methylene blue is much less inhibitory toward the acid-fasts and other microörganisms. Only the timothy bacillus is as sensitive to methylene blue as to gentian violet. The smegma bacillus is also somewhat more sensitive to methylene blue than are the other acid-fasts. Staphylococcus aureus is very sensitive to methylene blue. A 1:1,000 dilution of methylene blue is necessary to inhibit the growth of *B. subtilis*.

The results of another set of experiments, using the more slowly growing acid-fasts along with K1, all grown on glycerol-agar media, are shown in table 3. On glycerol agar even a 1:1,000 dilution of either gentian

[3] *The sources of the acid-fast bacilli used in this report:* The timothy, grass and Mist bacilli, are those of Moeller. The leprosy bacilli, so called, were from a strain isolated by Duval from a leprous lesion. The smegma, frog and avain bacilli were from old laboratory strains, the original history of which was not definitely known.

TABLE 2

Inhibition of growth on plain agar by gentian violet and methylene blue

TIME OF GROWTH	DYES	DILUTIONS	K1	TIMOTHY BACILLUS	GRASS BACILLUS	MIST BACILLUS	SMEGMA BACILLUS	B. COLI	B. PRODIGIOSUS	B. SUBTILIS	STAPHYLOCOCCUS AUREUS
2 days	G.V.	1:100,000	+++	+++	+++	+++	+++	+++	+++	− − −	− − −
		1:10,000	− − +	− − −	− − −	− − −	− − −	+ + +			
		1:5,000	− − −	− − −	− − −	− − −	− − −	+ + +			
		1:1,000	− − −	− − −	− − −	− − −	− − −	− − −	− + +	− − −	− − −
	M.B.	1:100,000	+++	+++	+++	+++	+++	+++	+++	+++	− − −
		1:10,000		− − −						+ + +	
		1:5,000		− − −						+ + +	
		1:1,000	+ + −	− − −	+ + +	+ − +	− − +	+ + +	+ + +	− − −	− − −
5 days	G.V.	1:100,000	+++	+++	+++	+++	+++	+++	+++	− − −	− − −
		1:10,000	− − +	− − −	+ − −	− − −	− − −	+ + +			
		1:5,000	− − −	− − −	− − −	− − −	− − −	+ + +			
		1:1,000	− − −	− − −	− − −	− − −	− − −	− − −	− + +	− − −	− − −
	M.B.	1:100,000	+++	+++	+++	+++	+++	+++	+++	+++	− − −
		1:10,000		− − −						+ + +	−
		1:5,000		− − −						+ + +	
		1:1,000	+++	− − −	+++	+++	+ − +	+++	+++	− − −	− −

G.V. = gentian violet. M.B. = methylene blue.

TABLE 3

Inhibition on glycerol agar, three slants of which were used

		K1		AVIAN		LEPROSY BACILLUS		FROG BACILLUS	
		2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
1:100,000	G.V.	+++	+++	− − −	− − −	+ − +	+ − +	− − −	− − −
	M.B.	+++	+++	+++	+++	+++	+++	+ − +	+ − +
1:10,000	G.V.	+++	+++	− − −	− − −	− − −	− − −	− − −	− − −
	M.B.	+++	+++	+++	+++	− + +	+++	− − −	− − −
1:5,000	G.V.	+++	+++	− − −	− − −	− − −	− − −	− − −	− −
	M.B.	+++	+++	+ +	+ +	+ − −	+++	− − −	− −
1:1,000	G.V.	+++	+++	− − −	− − −	− − −	− − −	− − −	− − −
	M.B.	+++	+++	− − −	− − −	− − −	− − −	− − −	− − −

G.V. = gentian violet. M.B. = methylene blue.

violet or methylene blue does not inhibit K1 growth. The frog bacillus is very sensitive to both gentian violet and methylene blue, a strength of 1:100,000 of these dyes entirely inhibiting its growth. The avian bacillus is as sensitive to gentian violet as is the frog bacillus, but it is a little more resistant to methylene blue. About the same thing is true for the leprosy bacillus, but this microörganism is not so sensitive as the other two to the dyes. The behavior of these three microörganisms toward gentian violet was most striking. They all grew well on the plain glycerol-agar side, but their growth stopped some distance from the septum, to indicate that they do not even approach where there is any trace of gentian violet. Therefore, they behave as do ordinary gram-positive microörganisms. The bacillary suspension was inoculated on the dyed part as one straight line coming from the plain-agar side. Some investigators have reported that they inoculated only on the plain-agar side, and observed whether the growth would cross the septum into the dyed part. But this was not the original direction of Churchman for his divided-plate method. The dishes inoculated with frog bacilli were also incubated. This was done after it was confirmed that frog bacilli grew in the incubator as well as at room temperature. However, some dishes inoculated with frog bacilli were kept at room temperature as controls.

2. Bactericidal Experiments

Bactericidal experiments were also tried with gentian violet and methylene blue on the various microörganisms used in the above inhibitory experiment. In this work the factor of time for the exposure of microorganisms to the dyes was constant while the strengths of the reagents were varied. The microörganism experimented on was brought into contact with the dyes in the following manner:

A certain amount each of 1.5:100, 1:100, and 1:5,000 solutions of gentian violet and methylene blue was made to serve as stock solutions. From 1:200 to 1:1,000,000 dilutions of both dyes were made, in general, by combining the proportionate amounts of the stock solutions and the broth cultures of various microörganisms, making the total 10 cc. in each tube. The 1:200 and 1:500 solutions of methylene blue, in the experiment shown in table 4, B, were made by weighing out the solid dye into test tubes and dissolving it with the culture broth of various microörganisms. This dye, 0.1 and 0.2, respectively, of methylene blue, had been heated in an electric hot-air oven at 110°–130°C. for one hour

TABLE 4

Bactericidal experiment on plain agar

		(A) GENTIAN VIOLET (GRÜBLERS)										(B) METHYLENE BLUE (RECT. N. EHRLICH)									
		K1		B. Prodigiosus		B. subtilis		B. coli		Staph. aureus		K1		B. prodigiosus		B. subtilis		B. coli		Staph. aureus	
		2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
1:100	*B.*											−	+			−	−	+	+	−	−
	a											+	+			+	+	+	+	−	−
	b											+	+			+	+	+	+	−	−
	c											+	+			+	+	+	+	−	−
1:500	*B.*											+	+	+	+	+	+	+	+	−	−
	a											+	+	+	+	+	+	+	+	−	−
	b											+	+	+	+	+	+	+	+	−	−
	c											+	+	+	+	+	+	+	+	−	−
1:1,000	*B.*	−	+	+	+	−	−	+	+	−	−	+	+	+	+	+	+	+	+	−	−
	a	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−
	b	+	+	+	+	−	−	+	+	−	−	+	+	+	+	+	+	+	+	−	−
	c	+	+	+	+	−	−	+	+	−	−	+	+	+	+	+	+	+	+	−	−
1:5,000	*B.*	−	+	+	+	−	−	+		−	−	+	+	+	+	+	+	+	+	−	−
	a	+	+	+	+	−	−	+		−	−	+	+	+	+	+	+	+	+	+	+
	b	+	+	+	+	+	+	+		−	−	+	+	+	+	+	+	+	+	−	+
	c	+	+	+	+	+	+	+		−	−	+	+	+	+	+	+	+		−	−
1:10,000	*B.*	−	+	+	+	−	−	+		−	−	+	+	+	+	+	+	+	+	+	+
	a	+	+	+	+	+	+	+		−	−	+	+	+	+	+	+	+	+	+	+
	b	+	+	+	+	+	+	+		−	−	+	+	+	+	+	+	+	+	−	−
	c	+	+	+	+	+	+	+		−	−	+	+	+	+	+	+	+	+	+	+
1:50,000	*B.*	+		+	+	+		+		−	−	+	+	+	+	+	+	+	+	+	+
	a	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
	b	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
	c	+		+	+	+		+		−	+	+	+	+	+	+	+	+	+	+	+
1:100,000	*B.*	+		+	+	+		+		−	−	+	+	+	+	+	+	+	+	+	+
	a	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
	b	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
	c	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+

TABLE 4—*Concluded*

		(A) GENTIAN VIOLET (GRÜBLERS)										(B) METHYLENE BLUE (RECT. N. EHRLICH)									
		K1		B. prodigiosus		B. subtilis		B. coli		Staph. aureus		K1		B. prodigiosus		B. subtilis		B. coli		Staph. aureus	
		2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
1:500,000	*B.*	+		+	+	+		+		−	+	+	+	+	+	+	+	+	+	+	+
	a	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	b	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	c	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
1:1,000,000	*B.*	+		+	+	+		+		+	+	+	+	+	+	+	+	+	+	+	+
	a	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	b	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	c	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
Control	*B.*	+		+	+	+		+		−	+	+	+	+	+	+	+	+	+	+	+
	a	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	b	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+
	c	+		+	+	+		+		+		+	+	+	+	+	+	+	+	+	+

B. = broth. a, b, c, = three slants of plain agar.

TABLE 5

Bactericidal on plain broth and agar

		6 HOURS IN GENTIAN VIOLET (GRÜBLER'S)															
		K1		Timothy bacillus		Grass bacillus		Mist bacillus		Smegma bacillus		B. subtilis		B. coli		B. prodigiosus	
		2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
1:200	B.	−	+	−	+	−	+	−	+	−	+	−	−	−	−	−	−
	A.	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
1:500	B.	−	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+
	A.	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
1:1,000	B.	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
	A.	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+

B. = broth. A. = agar.

before being dissolved in the 10 cc. of broth culture. For the experiments shown in table 4, B, and table 5 two-day-old cultures were used, while three-day-old cultures were used for the other experiments.

The degree of development of the microörganisms used in the culture

TABLE 6

Bactericidal on glycerol media

DILUTION	DYES	MEDIA	K1		AVIAN BACILLUS		LEPROSY BACILLUS		FROG BACILLUS	
			2 days	5 days	2 days	5 days	2 days	5 days	2 days	5 days
1:200	G.V.	Glyc. B.	−	+	−	−	−	−	−	−
		Glyc. A. 1	+	+	−	−	−	−	−	−
		Glyc. A. 2	+	+	−	−	−	−	−	−
		Glyc. A. 3	+	+	−	−	−	−	−	−
	M.B.	Glyc. B.	−	+	−	−	−	−	−	−
		Glyc. A. 1	+	+	−	−	−	−	−	−
		Glyc. A. 2	+	+	−	−	−	−	−	−
		Glyc. A. 3	+	+	−	−	−	−	−	−
1:500	G.V.	Glyc. B.	+	+	−	−	−	−	−	−
		Glyc. A. 1	−	+	−	−	−	−	−	−
		Glyc. A. 2	−	+	−	−	−	−	−	−
		Glyc. A. 3	+	+	−	−	−	−	−	−
	M.B.	Glyc. B.	−	+	−	−	−	−	−	−
		Glyc. A. 1	+	+	−	−	−	−	−	−
		Glyc. A. 2	−	+	−	−	−	−	−	−
		Glyc. A. 3	+	+	−	−	−	−	−	−
1:1000	G.V.	Glyc. B.	+	+	−	−	−	−	−	−
		Glyc. A. 1	+	+	−	−	−	−	−	−
		Glyc. A. 2	+	+	−	−	−	+	−	−
		Glyc. A. 3	+	+	−	−	−	+	−	−
	M.B.	Glyc. B.	−	+	−	−	−	−	−	−
		Glyc. A. 1	−	+	−	−	−	+	−	−
		Glyc. A. 2	+	+	−	−	−	+	−	−
		Glyc. A. 3	+	+	−	−	−	+	−	−

Glyc. B. = glycerol broth. Glyc. A. = glycerol agar, three slants of which were used.

broth varied greatly. For instance, judged by the degree of turbidity of their broth cultures, *B. prodigiosus* grew more than five times as much as the culture of *Staph. aureus* in the experiment reported in table 4, B. In the experiment of table 6, K1 multiplied more than six times and

the leprosy bacillus more than three times as much as the avian tubercle bacillus. In the experiment of table 5, the degree of growth of the microörganisms used was, as follows, according to the degree of turbidity of their broth cultures:

K1	*B. timothei*	*B. graminis*	*B. mist*	*B. smegmatis*
++++	+++	+++	+++	++++

B. subtilis	*B. coli*	*B. prodigiosus*
+++++	+++++	+++++

To control the experimental results, the broth tubes with bacterial growth were made approximately equal in regard to their turbidity. This was effected by diluting the better-growing broth culture with broth of the same stock as was used for the culture, and matching them with the least-growing culture broth in the same set of experiments. The above procedure was adopted in the experiments of tables 5 and 6. Then, thus standardized, the culture broths were combined with the solutions of the dyes to make up the desired various strengths as mentioned above.

After all tubes in one set of experiments were dyed, they were incubated together for two hours. Afterward the incubation transfers were made with a large platinum loop. One broth tube and three (one in the experiment of table 5) agar slants were inoculated with three loopfuls from each dyed broth tube. The whole procedure was done in order, and with such care that the time of contact of bacilli with the dye outside the incubator was about the same for all tubes in any one set of experiments; the broth culture that stood longest at room temperature after being dyed, and before being placed into the incubator, was the first to be transferred on to new media after removal from the incubator. In table 4, A, the duration of exposure of the bacilli to the dye before and after the incubation was three hours and a half, making a total of five hours and a half, including the period of incubation. However, in the transfer to new broth, the procedure was delayed, so that the duration of contact of bacilli with the dye at room temperature was five hours and a half, making the total for the broth set seven hours and a half. In table 4, B, the exposures to the dye were six and eight hours, respectively, for the agar and broth cultures. In tables 5 and 6, the experiments were carried out more satisfactorily and quickly, the total exposures to the dye being six hours for the former experiment, that is, of table 5, and five hours for the latter. In table 4, experiment A,

three drops were transferred on to new media from the 1:1,000 set, while one loopful was used for all the other transfers. The broth tubes were inoculated with three loopfuls. In table 4, experiment B, three drops were used for all transfers to agar and broth except for the 1:1,000 set, when only one loopful was used. In the other two experiments three loopfuls were transferred in every case. The results were recorded at various periods for twenty days during incubation of the transplanted cultures. However, as these results varied but little after five days of incubation, only the results taken after two days and five days are given in the tables. When there was some noticeable change later than five days' incubation, it was considered in the tabulation. Although the degree of growth was not graded by the use of different signs in these tables, the results given for the two-day and five-day periods of incubation will show it adequately. The tabulation of the inhibitory experiment was done in the same way.

From these tables it is seen that for all microörganisms used in the above experiments gentian violet is more bactericidal than methylene blue except in one case in which these two dyes were tested on K1 using glycerol-agar media (table 6). The experiments of tables 4 and 5 deal with acid-fast, gram-positive and gram-negative microörganisms. A certain bactericidal action of the two dyes even against the fast-growing acid-fasts (which are also gram-positive) is shown in these tables. The bacillary suspensions used in these experiments were made rather heavy. In the experiment with slow-growing acid-fasts, using glycerol agar and glycerol broth, 1:200 to 1:1,000 solutions of the dyes were tried (table 6). Both gentian violet and methylene blue show a remarkable bactericidal effect on them. The growth of avian tubercle bacilli and frog bacilli is less constant than the others, and there were no control sets in the experiment dealing with them. However, the results given in the table look reasonable in the light of their behavior toward the two dyes in Churchman's divided plates. The considerable bactericidal action of gentian violet and methylene blue for leprosy bacilli is also demonstrated in the table.

The bactericidal power of gentian violet for gram-positive microorganisms is high, destroying fairly completely, at a 1:1,000 dilution, such a fast-growing germ as *B. subtilis* in a thick suspension. *Staphylococcus aureus* was thoroughly destroyed at a 1:10,000 dilution. Table 5 is the result of experiments more carefully conducted than those reported in table 4. A 1:200 dilution of gentian violet destroyed such

gram-negative bacilli as *B. prodigiosus* and *B. coli*. The action of methylene blue is comparable to that of gentian violet, only being milder.

DISCUSSION

It is to be understood that the technique of the inhibitory and bactericidal experiments was entirely different. In the above bactericidal experiments the factor of time for exposing bacteria to the reagent was constant. The microörganisms were with various strengths of gentian violet and methylene blue for a definite time, and were then transferred to new broth or agar slants. Therefore, the microörganisms, transferred on to new media, carried some reagent with them which, in turn, acted more or less as an inhibitory reagent against the microörganisms. In the inhibitory experiments a certain amount of microörganisms was streaked on the media which had been permeated with the reagent. Consequently, the inoculated microörganisms were constantly under the inhibitory influence of the reagent. In view of this, we might compare the inhibitory and bactericidal actions of a reagent on the microorganisms. The inhibitory action of gentian violet for gram-positive bacilli is great, 1:100,000, the highest dilution tried, completely inhibiting the growth of both *B. subtilis* and *Staphylococcus aureus*, while its bactericidal power against *B. subtilis* is around 1:1,000, and is 1:10,000 against Staphylococcus. Gram-negative microörganisms are shown in tables to be very much less influenced by gentian violet than are the gram-positives; a strength of 1:1,000 is necessary for any remarkable inhibitory action, and a 1:200 dilution is required to kill them. The fast-growing acid-fast saprophytes, including K1, timothy, grass, mist and smegma bacilli, are inhibited on plain agar by 1:10,000 of gentian violet, while the death of these microörganisms begins to be effected by a solution of 1:500. The slow-growing acid-fasts, along with K1, were tested, using glycerolated media in both the inhibitory and bactericidal experiments (tables 3 and 6). K1 was affected approximately to the same extent by both gentian violet and methylene blue in glycerol media. The growth of K1 was not entirely inhibited by a 1:1,000 dilution of gentian violet or of methylene blue in glycerol agar. Avian tubercle bacilli and frog bacilli were killed by either dye in a strength of 1:1,000, the highest dilution used for the bactericidal experiments, and leprosy bacilli were greatly affected by the same strength of the dyes. Avian tubercle bacilli and frog bacilli were entirely inhibited by 1:100,000 of gentian

violet, the highest dilution tried, and leprosy bacilli by 1:10,000. The action of methylene blue is similar in almost every way to that of gentian violet in the inhibitory and bactericidal experiments except that it is much less powerful.

The susceptibility of frog, avian and leprosy bacilli to gentian violet is striking. This specific action of gentian violet against the slow-growing acid-fasts is presumably due to a certain chemical factor characteristic of these microörganisms. The cultures on glycerol-agar slants of these acid-fasts are slimy, and their acid-alcohol-fastness is not high; that of the frog bacillus is especially very poor. According to the chemical analysis of Long and Campbell, the three acid-fasts mentioned above have approximately an equal saponification number, that is, around 190, which is much different from other fast-growing acid-fast saprophytes and the slow-growing virulent tubercle bacilli. Their percentages of non-saponifiable lipoid in the total lipin are approximately the same, being around 30, which is much different from those of the other acid-fasts. Long and Campbell also report that the avian bacillus and the leprosy bacillus gave a lipin product which was not soluble in petroleum ether, this point being different from that of other acid-fasts (*Wells, DeWitt, and Long, The Chemistry of Tuberculosis, 1923, pp. 113–114*).

There stand out in the above experiments several prominent points. First is the influence of the factor of the culture medium on which these experiments were conducted. The experiments using plain agar gave different results from those in which glycerol agar was used. The use of plain agar served to magnify the differences in the reactions of the microörganisms to the dyes, while it also helped to bring out the specific affinity of a microbe to a reagent better if the richer media are used. Second is the difference in the inhibitory and bactericidal action of a dye. Third is the difference in action of gentian violet and methylene blue; and fourth, a certain specific action of a dye on certain microorganisms. Methylene blue, in the inhibitory experiments, affected timothy bacilli to the same extent as did gentian violet, although the latter is generally superior to the former in antiseptic power. A specific nature in the chemistry or metabolism of timothy bacilli which could be correlated with this susceptibility to methylene blue has not yet been pointed out. Fifth is the striking effect of both gentian violet and methylene blue on the slow-growing acid-fasts. This does not mean that the dyes would act very strongly on the more slowly growing acid-

fast human and bovine tubercle bacilli. On the contrary, about 1:10,000 dilution of gentian violet in glycerol-broth-egg media seems to have little inhibitory action on these virulent acid-fasts (*Petroff, Bulletin of the Johns Hopkins Hospital, 1915, xxvi, 276*).

SUMMARY AND CONCLUSIONS

1. In this article, continuing the work reported in the first paper on the same subject, the nature of the non-acid-fast element in K1, a very weakly virulent strain of tubercle bacillus, was studied. The purpose was to eliminate the possibilities of symbiosis or a mutation strain in this culture, by employing sodium hydroxide and gentian violet. These substances were expected to separate the acid-fast from the non-acid-fast element, or gram-positive from gram-negative microörganisms. However, the acid-fast element was not left entirely acid-fast after digestion of the non-acid-fast element of K1 by sodium hydroxide. Nor was the gram-positive or acid-fast element eliminated by the inhibitory or bactericidal action of gentian violet. Therefore, the two elements in K1 strains must be of the same culture without any contamination or development of a mutation strain. Further, it became probable, after having compared the tinctorial nature of younger and older growths of K1 and virulent strains of tubercle bacilli and, also, from the success of some reduction of acid-fastness of K1 by rapid transfers reported in my previous paper, that the youngest stage of acid-fasts is non-acid-fast. This period of non-acid-fastness must be very short, judging from the small number of non-acid-fast microörganisms in the virulent culture and from the reported impossibility of a complete disappearance of acid-fastness of K1 by the rapid transfers. The speed of propagation of virulent tubercle bacilli was grossly measured as a means of explaining this point. It was done by taking a uniform weight for inoculating media, and then weighing the total growth of the slants when three weeks old. We might say that it takes five days for a tubercle bacillus to double its weight, or to make one cell division. The non-acid-fast stage must be very short in this relatively long period.

2. In the experimental part on the inhibitory and bactericidal action of gentian violet and methylene blue, which was conducted in connection with the above studies on the non-acid-fast element of K1, the following points may be noted:

a. As a rule, gentian violet affects more markedly than methylene

blue not only gram-positive bacilli, but also gram-negative and acid-fast microbes. The exception of timothy bacilli to this rule is to be noted. A much stronger solution of the dyes is required for bactericidal than for inhibitory action. The kind of media used makes a great difference in the results. Both poor and rich culture media are serviceable to make the results more distinct. The action of gentian violet and methylene blue on slow-growing acid-fasts, that is, the frog, avian tubercle, and leprosy bacilli (Duval), is striking. These bacilli seem to have something in common in their chemical nature. The poor acid-fastness of the frog bacillus is remarkable. Gram-positives, gram-negatives, fast-growing acid-fasts, and slow-growing acid-fasts used in these experiments grouped themselves according to their reaction to gentian violet and methylene blue.

b. Gentian violet inhibited gram-positive bacilli and slow-growing acid-fasts in a 1:100,000 dilution, the highest dilution tried in this work. Fast-growing acid-fasts were inhibited by 1:10,000 of gentian violet in plain agar, but they were not so much influenced by this dye in glycerol agar. A 1:1,000 dilution inhibited the growth of gram-negative bacilli. The bactericidal action of gentian violet also varied for the different groups of microbes. Gram-positives and slow-growing acid-fasts were destroyed by a 1:1,000 dilution; gram-negatives by 1:200. Fast-growing acid-fasts were not much affected by these strengths of gentian violet.

c. The effect of methylene blue on these microörganisms while similar, is very much milder.

Horace John Howk

1878-1926

Horace John Howk, Assistant Medical Director of the Metropolitan Life Insurance Company, and Physician-in-Charge of the Company's Sanatorium at Mount McGregor, New York, died on April 8, 1926, at the Johns Hopkins Hospital. His death has caused the deepest grief and a sense of irreparable loss among the officers, patients and a host of close personal friends. He had appeared to be in even better than his usual good health when he came to New York in the latter part of January to attend the Company's annual convention of managers, but fell ill at his hotel on the day of his arrival with what appeared to be an acute arthritis. Later he was cared for at the New York Hospital, until the fever and painful symptoms had disappeared, and then went to Florida to finish his convalescence in that sunshine which he loved so much. His recovery there appeared to be progressing favorably for a while, but suddenly the old and new symptoms returned in greater intensity and then, at his earnest desire, he was brought to the Johns Hopkins Hospital, where he passed away three days later.

Doctor Howk was born in Ontario County, New York, in 1878. He finished his academic studies at the University of Rochester in 1904, and received his doctorate in medicine from the University of Michigan in 1907. He was appointed Instructor in Clinical Pathology at Michigan in 1907 and a year later, after he had suffered with long and severe recurring mastoiditis, went to Loomis Sanatorium as the proper place to regain his health and to specialze in the care and treatment of the tuberculous. There he worked as Associate Physician with Dr. H. M. King for three years. Leaving Loomis in February, 1911, it was in the following July that he was asked, and accepted the invitation, to direct the development and to undertake the management of the Metropol tan Life Insurance Company Sanatorium at Mount McGregor.

The achievements of Doctor Howk are well known to all those interested in tuberculosis work in this country and abroad. He was recognized not only for his exceptional abilities in selection and organization of his staffs and in his business administration, but was dearly loved

Pach Photo

Horace J. Howk
1878-1926

by the thousands for his fine qualities of mind and heart. He was gentle, kindly and unaffected. He was knowing, able and courageous. Of his own courage and faith he gave to others. His ideals in life and work stimulated and guided his associates. The thousands of Metropolitan employees who knew him at Mount McGregor were devoted to him for the healing they found in his sound judgment and in his sympathetic counsel. He knew and called each patient by name and the Sanatorium under his management did not have the atmosphere of an institution but rather of a home, of which each patient was an individual member. He blazed the way for the successful treatment of varied diseases in a Sanatorium that was purposed primarily for the care of the tuberculous.

Doctor Howk had many community interests at heart and his influences were exerted far beyond the groups on Mount McGregor. He was President of the Saratoga County Medical Society from 1915 to 1917, and for years was President of the Saratoga County Health and Tuberculosis Committee. For two years he lectured on tuberculosis in the Albany Medical School. His services as a consultant in critical cases of illness were eagerly sought by physicians and patients throughout the neighboring counties, even at great distances. He was a member of the Board of Managers of the Glens Falls Hospital and a prominent trustee of Skidmore College in Saratoga Springs. He was a member of Alpha Delta Phi, Nu Sigma Nu and Sigma Xi. In 1912 he married Lois Hollister of Rochester, who survives him.

He lived wisely, happily and to great purpose. He loved and understood people and was beloved by them. To an unusual degree he knew and appreciated books and prints and music. An ardent lover of nature, he spent many happy hours in the woods and on the streams with his dogs, his gun and his rod. His death has brought sorrow to his friends, and a great loss to the organization with which he worked and to medicine in America.

The work of the Metropolitan Life Insurance Company Sanatorium at Mount McGregor, as established and carried on by him, will be his perpetual memorial.

A. S. K.

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